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# EFFECT OF INTRAVENOUS INJECTION OF SODIUM CHLORIDE ON THE DISTRIBUTION OF WHITE BLOOD CELLS IN THE PERIPHERAL CIRCULATION

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The factors governing the total numbers and the normal rhythm of the white blood cells in the peripheral circulating blood under normal, pathological and experimental conditions have been the subject of extensive investigation during the past forty years.

One phase of this subject which has received especial attention has been the interesting reaction which is produced by the intravenous injection of various substances, most of which have been proteins or protein derivatives. This reaction, consisting of a leucopenia developing within a few minutes after the injection and followed later by a leucocytosis, has been studied by many workers, among whom Roemer (1892), Werigo (1892), Goldscheider and Jacob (1894) and Arneth (1920) must be especially noted. This reaction of the white blood cells may be illustrated best by a typical experiment of Arneth's. He injected 10 cc. of a 10 per cent solution of peptone intravenously into a normal dog and found that there was a drop in the total number of white blood cells from a normal of 11,000 to 2,400 within ten minutes. Thirty minutes later the count began to rise slowly, and twenty-four hours after the injection it reached 12,400 cells per cu. mm. Among the substances which have been found to produce similar effects on the white blood cells are bacterial products, tissue extracts, egg white, sodium cinnamate, carmine, etc. With a single exception, so far as we have been able to determine, all the substances which have been found to produce this peculiar curve of leucocytic distribution have been of an organic nature. This exception is in the use of sodium chloride by

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Boyd (1913) and Bluemel and Lewis (1924). Boyd found a rise in the white cells from 9,500 to 15,400 following an intravenous injection of 40 ounces of 0.75 per cent sodium chloride into an adult human being, and five days later another injection of the same amount of saline resulted in a rise from 10,000 to 28,000 white cells. Bluemel and Lewis injected large amounts of physiological salt solution into normal adult human beings and found that after about six hours, there was an increase in the total

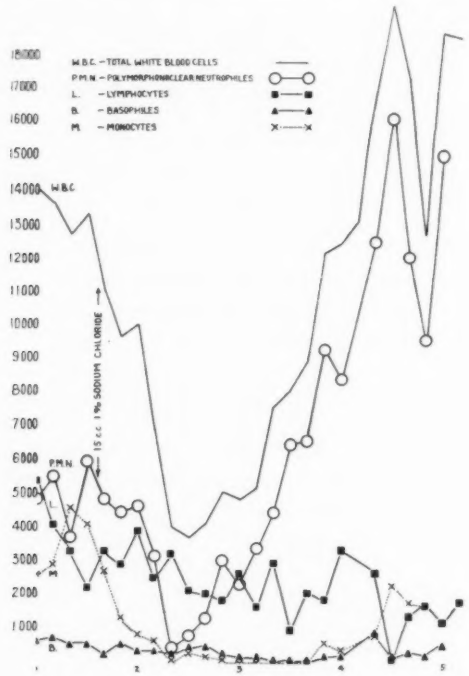


Chart 1. Curves showing the total white blood cells, polymorphonuclear neutrophils, lymphocytes, basophils and monocytes in experiments 50–83.

white count of 66 per cent, or from an average of 5,433 to an average of 9,050. On the other hand, in neither of these investigations was the blood of the subjects studied immediately after the injection, and no mention is made of any evidence of a leucopenic phase occurring before the leucocytosis.

In the course of a series of experiments on rabbits, in which we were studying the effects of many inorganic salts on the white blood cells, we found that if counts were made immediately following an intravenous

injection of a small amount of 1 per cent sodium chloride, there was a rapid fall in the white blood count followed by a rise which carried the count to the normal level or above. The fact that sodium chloride, which is a simple inorganic salt, normally present in relatively high concentration in the blood, gives the same reaction as complicated compounds of organic nature, indicates the possibility of its being a fundamental factor in the variations in numbers of the white blood cells. In order to analyze this reaction more carefully, we have carried out eight experiments on rabbits in each of which we injected a given amount of a solution of sodium chloride and have followed the reaction of the white blood cells by taking counts both before and after the injection at ten minute intervals. In six experiments 15 cc. of a 1 per cent solution were given, and in the other two, 10 cc. of a 2.5 per cent solution. Counts were continued at ten minute intervals for two and one-half to three and one-half hours. A total white blood count and a differential were made at each interval in six experiments. In two experiments only total white and total red cell counts were made. The supravital method of Sabin (1923) was used in making the differential counts. We have tried to maintain uniformity of technique in all of the experiments; one worker obtained all the blood and made the total white cell counts, while the other made all the differential counts and the red cell counts.

The results of the injection of sodium chloride on the total white counts were practically identical in all eight experiments. Chart 1 (expt. 50–83) has been included as an example of the numerical changes which occurred; the total number of each type of cell and of the total white blood counts are shown. The eosinophils and non-motiles have been omitted for the sake of simplicity. In this experiment four preliminary counts were made, which established the normal level of the total white blood cells at 13,560. The normal numbers of neutrophils, non-motiles, basophils, eosinophils, lymphocytes and monocytes, respectively, were also determined. It will be noted that the normal level of monocytes for this particular animal was somewhat high, otherwise the differential percentages of white cells were about the average of those usually found in rabbits. Following these normal counts, the rabbit was injected with 15 cc. of a 1 per cent solution of sodium chloride. The count immediately began to fall, and fifty-five minutes after the injection it reached its lowest point, 3,800. It remained relatively low for forty minutes, at the end of which time it had risen to 5,200 cells. Following this, it climbed rapidly, reaching its maximum of 19,500 eighty minutes later. The curve then varied somewhat but showed a decided tendency to drop.

It is evident that the neutrophils were chiefly involved in the leucopenia, in that they showed a corresponding diminution in numbers to that occurring in the total count, during this time, sufficient to account for the major

part of the leucopenia. It should be noted that they fell precipitately to their lowest level ten minutes earlier than the lowest white cell count was reached. This leucopenia of the neutrophils was maintained for thirty minutes, the count rising only slightly during this time. Following this there was again an anticipation of the change in numbers of the total white cells in a disproportionate rise of the neutrophils, which began twenty minutes earlier than the rise in the total white cells. The relatively greater rise in the numbers of neutrophils is shown in a closer approximation of the curve of their total numbers to the curve of the total white cells in the latter counts of the experiment, than in the normal preliminary counts. In this experiment they reached a height of approximately 10,000 cells above any of the normal counts. The basophils showed no marked change, other than a somewhat lower level of their numbers following the injection. The eosinophils were present in very small numbers and so had practically no part in the large variations which occurred. It is remarkable, however, that they fell to zero at the beginning of the leucopenia, and remained at a very low level for the eighty minutes following, after which they recovered somewhat but did not regain their original number before the end of the experiment.

The curve of the lymphocytes presented no striking changes and did not conform in any way to the curve of the total white cells. The lymphocytes, however, tended to slope downward, and at the end of the experiment they were approximately 2,000 cells lower than the normal level. It is seen, then, that while they played a small part in the leucopenia following the injection, they were not a factor in the rise of the total white cells to normal following the leucopenia, since they continued to decrease throughout the entire experiment. The curve of the monocytes is very interesting. The total number of monocytes in this animal was 3,630. They disappeared completely from the peripheral circulating blood in forty-five minutes after the injection and remained practically at zero for eighty minutes. They then rose gradually to approximately 1,400 and were present in slightly less than half of their normal numbers three and one-half hours after the injection. Thus the monocytes also had a considerable part in the leucopenia, but responded only very slightly in the compensatory phase of the curve which followed. In the other experiments the curves of the monocytes were essentially the same, but as their initial counts were much lower than in the one described above, any part they might have played in the leucopenia is negligible in comparison with the great variation in the total white cell count.

The non-motiles are noteworthy in that the injection of sodium chloride had little or no effect upon the normal rhythm of these cells. Every fifty to seventy minutes a few appeared in the peripheral circulating blood just as happened in normal rabbits. The highest count of non-motiles

obtained in any of these experiments was 1,562, and this number is not above that normally found in the blood of the rabbit. This finding is in contrast with the massive showers of non-motiles following the administration of other salts such as sodium citrate in rabbits, as reported by Beard and Beard (1927).

TABLE 1

*Showing the total numbers of white blood cells in the eight experiments*

| MINUTES | EXPERIMENTS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 42-62 | 43-65 | 46-78 | 49-76 | 50-83 | 51-84 | 52-85 | 33-86 |
| 80 | 8,520 | | | | | | | |
| 70 | 10,640 | | | | | | | |
| 60 | 10,160 | | | | | | | |
| 50 | 10,440 | 12,880 | | | | | | |
| 40 | 9,000 | 12,520 | 8,400 | | | | | |
| 30 | 8,920 | 8,480 | 8,480 | 12,840 | 14,160 | | 15,720 | 13,440 |
| 20 | 8,720 | 9,200 | 11,320 | 12,160 | 13,720 | 10,800 | 12,240 | 12,200 |
| 10 | 6,680 | 9,560 | 12,200 | 12,120 | 12,880 | 9,800 | 12,680 | 14,960 |
| 0 | 8,040 | 11,600 | 11,080 | 13,960 | 13,480 | 10,480 | 14,920 | 14,240 |
| | | | | Injection | | | | |
| 5 | 8,560 | 16,640 | 12,080 | 15,120 | 11,200 | 8,640 | 16,280 | 14,000 |
| 15 | 5,560 | 9,720 | 9,200 | 12,280 | 9,760 | 8,280 | 14,260 | 13,000 |
| 25 | 8,600 | 7,800 | 10,680 | 9,960 | 10,040 | 8,320 | 11,400 | 10,760 |
| 35 | 3,760 | 6,840 | 9,840 | 9,600 | 6,960 | 6,960 | 8,920 | 8,360 |
| 45 | 3,240 | 5,400 | 6,800 | 8,240 | 4,120 | 3,520 | 4,880 | 4,880 |
| 55 | 2,800 | 4,480 | 4,480 | 7,960 | 3,800 | 4,320 | 8,640 | 5,080 |
| 65 | 5,000 | 5,320 | 4,680 | 7,960 | 4,200 | 4,560 | 8,240 | 4,880 |
| 75 | 5,520 | 5,200 | 4,320 | 9,120 | 5,160 | 5,720 | 7,560 | 5,480 |
| 85 | 6,520 | 5,280 | 4,960 | 10,880 | 4,920 | 6,000 | 9,080 | 5,880 |
| 95 | 6,920 | 6,320 | 6,040 | 12,480 | 5,240 | 7,400 | 9,080 | 4,560 |
| 105 | | 8,080 | 8,840 | 13,400 | 7,560 | 10,440 | 10,360 | 5,800 |
| 115 | | 7,000 | 9,240 | 12,240 | 8,080 | 6,800 | 10,880 | 5,400 |
| 125 | 14,760 | 13,640 | 11,560 | 14,400 | 8,960 | 11,080 | 12,320 | 7,240 |
| 135 | 15,720 | 9,120 | 9,680 | 15,880 | 12,280 | 9,120 | 19,800 | 9,840 |
| 145 | 17,920 | 10,560 | 12,480 | 17,120 | 12,560 | 11,400 | 15,480 | 13,360 |
| 155 | 20,040 | 11,400 | 12,000 | | 13,200 | | 18,840 | 14,080 |
| 165 | | 11,640 | | | 17,040 | | 15,080 | 14,360 |
| 175 | | 13,000 | | | 19,640 | | | 29,720 |
| 185 | | 11,840 | | | 17,360 | | | |
| 195 | | 13,200 | | | 12,800 | | | |
| 205 | | 8,000 | | | 18,800 | | | |

Table 1 gives the total white cell counts of the eight experiments. From a study of this table it may be seen that there are six fairly well defined divisions in the curves. These are: 1, the establishment of a normal level;

2, a slight rise above or maintenance of the normal level in the first count following the injection; 3, a rapid fall to a very low level; 4, a period in which the count remains practically stationary; 5, a rapid rise to normal or above; and 6, large fluctuations in the curve with a general tendency to drop. Counts taken five minutes after the injection showed that the total number of white blood cells remained normal in five experiments, in spite of the fact that 15 cc. of fluid had been injected. In these animals there was a distinct drop in the counts taken ten minutes later. In three experiments, however, there was a prompt but very transitory rise in the total number of white cells during the first five minutes, the following count, ten minutes later, being within the normal range. The fall below normal in the second group, corresponding with that which took place fifteen minutes after injection in the first group, was thus twenty-five minutes after injection in the latter cases. The leucopenia in all the animals,

TABLE 2

*Showing the amount of injection given in each experiment, the average total white cell count, the average total white cell count during the period of leucopenia, the average drop from the normal level to the level in the leucopenia, and the maximum drop which occurred in each experiment*

| Experiment No. | 42-62 | 43-65 | 46-78 | 49-76 | 50-83 | 51-84 | 52-85 | 53-86 |
|---|---|---|---|---|---|---|---|---|
| Amount of fluid injected | 15 cc. 1% | 15 cc. 1% | 10 cc. 2.5% | 10 cc. 2.5% | 15 cc. 1% | 15 cc. 1% | 15 cc. 1% | 15 cc. 1% |
| Average, normal | 8,893 | 11,413 | 10,296 | 12,770 | 13,560 | 10,360 | 13,890 | 13,710 |
| Average, leucopenia | 3,950 | 5,136 | 4,610 | 8,320 | 4,513 | 4,530 | 4,880 | 5,126 |
| Average drop | 4,943 | 6,277 | 5,686 | 4,450 | 8,987 | 5,830 | 9,010 | 8,584 |
| Maximum drop | 6,093 | 6,933 | 5,976 | 4,810 | 9,760 | 6,840 | 9,010 | 9,150 |

however, was remarkably coincident and required fifty-five minutes in five cases and forty-five minutes in three cases to reach the lowest level. The actual reduction in the number of cells in all of the experiments was practically uniform (see table 2).

When the lowest level had been reached there was a period during which the count remained relatively constant. This period varied from forty to sixty minutes in seven of the experiments. In the eighth animal the duration of the period of leucopenia was markedly less. In this experiment, however, the rise following the leucopenic stage was slower than in the other animals, and the normal level was reached at approximately the same interval following injection. The rise in all of the experiments terminated with considerable variations in the white cell counts followed by a gradual decrease in numbers toward normal.

Two experiments were carried out in which both the total white and total red cells were counted at the same intervals, both before and after the

injection of sodium chloride. In these experiments the white cells showed the typical reaction of a leucopenia followed by leucocytosis after the injection. The red cells, on the other hand, were scarcely affected and maintained a perfectly normal smooth level during the period of the variations in the white cells. The only irregularity in the red cell curve was manifested immediately following the injection when the curve dropped slightly and in the next count was slightly elevated. After this, it main-

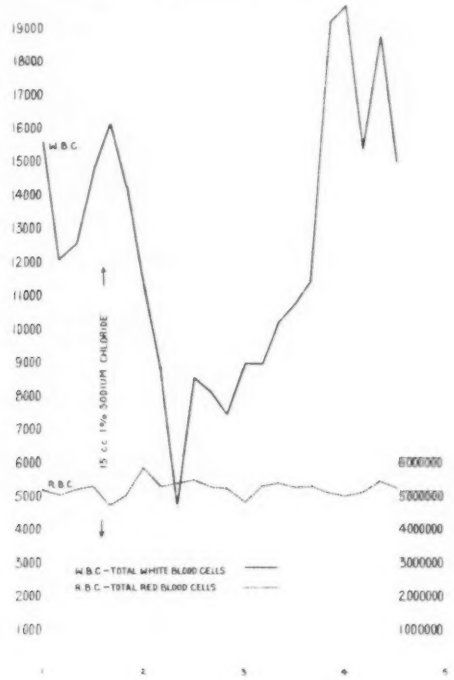


Chart 2. Curves showing total white blood cells and total red blood cells in experiments 52–85.

tained its smoothness to the end of the experiment. The stability of the red cell curve, which occurred in both of these control experiments, thus rules out any possibility of the variations in the white blood cells being the result of differences in the blood volume after the injection. One of these experiments is shown in chart 2.

When one considers the remarkable similarity of action on the white cells of the large group of organic substances which have been studied in

this connection, it seems very difficult to assume that such uniformity could be the case were there not some "common denominator" in this widely diversified group. That this common factor is not of a chemical nature is quite obvious, and therefore it must be sought for in some physical similarity or in some secondary effect. The results which we have obtained by injecting rabbits with sodium chloride solution correspond very well in type with the experiments reported in the literature, but they do apparently differ rather markedly in duration, since we have found a return to normal within three to five hours, whereas in the experiments with injections of proteins, etc., twenty-four hours were usually required for the return to normal. Whether there is a specific difference in the two experimental procedures or whether it is due to the failure of the workers studying proteins, etc., to count their animals at sufficiently close intervals, we can not say at present. This question is now being studied in a series of experiments which will be reported later.

The similarity in the effects on the white blood cells of the intravenous injection of the organic substances mentioned above and of sodium chloride, suggests the possibility that some disturbance of the salt balance in the body might be the modus operandi of the various unrelated organic substances which have been found to produce this effect. The ubiquity of sodium in the tissues and its fundamental importance in the physiological processes of the organism, makes this hypothesis a tenable one. However, from the experiments reported here, it is possible to say only that sodium chloride will produce an effect on the distribution of the white blood cells in the peripheral circulation which is entirely similar to and as pronounced as that produced by the many organic substances which have been studied.

## SUMMARY

Eight rabbits, previously standardized, were injected intravenously with 10 or 15 cc. of dilute sodium chloride solution. The results were uniform and were as follows:

1. Following injection there was a rapid fall in the white blood cell count to less than half of the average normal count. This leucopenia was maintained for from 30 to 50 minutes, following which there was a rapid rise to normal or above.

2. The polymorphonuclear neutrophils were chiefly involved in the changes produced, and showed corresponding variations in total numbers.

3. The polymorphonuclear eosinophils followed the same type of curve but were in such small numbers that they had practically no part in the large variations.

4. The lymphocytes fell gradually during each experiment.

5. The monocytes fell to zero with the beginning of the leucopenia, and

remained so until after the total white cells had become normal or above, at which time the monocyte curve began to rise.

6. The red blood cells showed no change.

7. The results obtained following the injection of sodium chloride are quite similar to those previously described as resulting from proteins, bacterial products, etc. This relationship is discussed.

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# COLOR AND FORM DISCRIMINATION IN THE PERIPHERY OF THE RETINA

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Purkinje was first to call attention to the fact that sensitivity to light has a wider retinal extension than sensitivity to color and that the various colors have retinal zones of different extension. Since his time a great deal of work has been done on this subject. A good historical sketch of the work on peripheral color vision may be found in Baird's monograph on *The Color Sensitivity of the Peripheral Retina* (1905). Practically all the workers in this branch of optics considered the field of vision for white light as coinciding with that of general visual sensibility, and they therefore limited their investigations to such colors as blue, yellow, red and green. Yet insofar as ability to discriminate colors is concerned white is as much an individual color as any of the above, and it appeared to us as interesting to find how far out from the fovea we would still be able to discriminate between white and the other colors. We were led to this work partly because of the results obtained by Piéron and one of us in a study on the speed of establishment of the luminous sensation in the fovea by the action of white light as well as by color excitations (Kleitman and Piéron, 1924, 1925).

It is now universally accepted that the cones are the retinal elements responsible for color vision, whether the colors are perceived in the central or the outlying regions of the retina. Since the cones become less and less dense as we go from the center to the periphery, it occurred to us that by investigating the ability of the peripheral regions of the retina to distinguish the various colors *and white* we might detect any differences, should such exist, much easier than by studying the extremely dense cone region in the center of the retina. When we examined the literature for data on the extent of the visual fields for various colors as obtained by one or another form of perimeter, we found a lack of agreement among the various investigators who studied this subject. Aubert (1865) made a very thorough study of the visual fields for colors, using a black background in one case and a white one in the other. He employed colored paper squares whose sides varied from 1 to 16 mm., and found that the larger the square the farther out it could be seen, but not to the same extent in all directions.

The squares could be seen farther out in the temporal half of the visual field than in the nasal half.

The average (for eight meridians) distance from the fovea for the 4 mm. squares on a black background was as follows: blue, 54°; red, 42°; yellow, 40° and green, 35°. Landolt (Snellen and Landolt, 1874) describes experiments Donders and he performed using direct sunlight, which was entering into a dark room through a narrow slit and was made to fall on pieces of colored paper 1 cm square. In the outer meridian of the visual field they could see all colors at 90° from the fovea. However, using ordinary illumination Landolt (quoted from Baird, 1905) found that the fields for different colors are not the same. Charpentier (1883), studying the outer meridian of the visual field, confirmed the findings of Landolt by a slightly different method.

Baird (1905) reinvestigated this subject very thoroughly, and came to the conclusion that "the position and extent of the color zones are not fixed but variable," and that "their area in any given case depends upon the momentary condition of retinal adaptation, and upon the brightness and saturation of the stimuli employed." Using a Hellpach perimeter where the stimulus is a light coming from a lantern after passing through colored gelatine, he found in five subjects with dark adapted eyes that the fields for blue and yellow are coextensive and larger than the fields for red and green, also coextensive.

Ferree and Rand (1920) found that the far periphery of the retina is deficient but not blind to blue, red and yellow, and that with stimuli of sufficient intensity the limits of these colors coincide with the limits of light vision. The peripheral blindness for green, however, was absolute.

Engelking (1921), employing spots of four different sizes, and investigating four meridians, concludes, like Baird, that the fields for blue and yellow, and for red and green are coextensive, and that the former are larger than the latter.

Finally, Peter (1926) gives, as the average for four meridians, for 5 mm. spots, for blue, 48° from fovea, red, 33°, green, 24°, and quotes de Schweinitz, for the same colors, but 10 mm. spots, as giving blue, 56°, red, 45°, and green 33°. There is then an agreement between Peter and de Schweinitz that the fields for red and green are not coextensive and that the field for red is larger than that for green.

The above mentioned papers represent but a few of the contributions to the subject of peripheral color vision, but they are representative. It will be noted that there is a complete agreement between the results of Landolt and Charpentier, Baird and Engelking, Peter and de Schweinitz.

We performed two series of experiments: first, on color discrimination, and second, on form discrimination.

FIRST SERIES. Aware of the discrepancies in the results obtained by

others, we decided to rule out the question of luminosity by adding a neutral grey and white to the four fundamental colors we started out to investigate. It may be argued that grey as well as white are not colors. But looking at a grey or a white spot with the foveal retina we can recognize them as such and distinguish them easily from blue, red, etc. The question we wanted answered was how far outward from the fovea will we still be able to distinguish between green and grey, or between white and yellow as well as we can by making them fall on the fovea.

*Method.* For mapping out the visual fields we used a McHardy recording perimeter. The tests were performed in a white walled room illuminated by sunlight. The walls dispersed the sunlight and no shadows fell on the perimeter. The stimuli consisted of colored paper sectors in a disc 5 cm. in diameter which could be made to move in and out along the curved band of the perimeter, 5 cm. wide, by means of a thumb screw placed behind a central shield that held a small ivory colored button on which the eye was fixed. The radius of curvature of this band was 35 cm., and that was the distance of the colored paper from the eye at all times. The disc with the colored sectors was covered by a black disc which had a variable circular opening, and which could be rotated so as to expose one or another of the colored sectors. We tried several apertures, and finally adopted two, one of 5 mm. and another of 10 mm. Thus our stimuli consisted of white, grey, blue, green, yellow and red discs of the above mentioned diameters, and they were moved in from the periphery on a black background and without being touched. The subject was to name one of the six colors as it was moved from the periphery to the center as soon as he was sure that he recognized it. The aperture was shifted and the color-stimulus changed at the very periphery, and while the subject looked away. The visual field was mapped out in the same test for all six colors, which were presented without definite order. In a certain definite meridian (or half-meridian, as some prefer to designate it) one or more, but never all six colors were tested, so that generally it was necessary to go around the circle several times before all the limiting points were charted. A record was kept of all colors named wrong. At first we studied 24 meridians, or every 15 degrees, but in our later experiments we found that by testing only 12 meridians we obtained very smooth perimeters. The smoothness of the line that connected the various points was, for us, an indication of lack of fatigue at the end of the experiment as well as of the value of the results. In our early experiments the perimeters obtained were irregular, having a star-shaped or zigzag outline, and when superimposed the perimeters for various colors crossed and recrossed each other. After a period of training, which consisted in learning to keep the eye on the point of fixation while viewing the colored disc in the periphery of the field of vision, we began to obtain smoother and smoother outlines

for the fields for the different colors. It is for this reason that we did not extend our observations to many subjects, even though it would be desirable to confirm the results we obtained on other individuals. We ourselves acted alternately as observer and subject, and only a few tests were made on a third subject, who was a careful and trained observer. Only one eye, right or left, was used for all tests. On some occasions we used only three or four colors, telling the subject about it. The subject was thus aware at all times of the various possibilities in colors that he was called upon to distinguish.

*Results.* We found, as did Aubert and others, that the limit of the visual field for different colors is different in one meridian from what it is in another.

TABLE 1

*The average extension of the visual fields for colors, in degrees from the fovea, based on figures for extension along twelve meridians, obtained with one six-color and two three-color discrimination tests*

| COLOR | 10 MM. DISCS | | | 5 MM. DISCS | |
|---|---|---|---|---|---|
| | Subject K | Subject L | Subject B | Subject K | Subject B |
| Blue | 47 | 46 | 42 | 37 | 32 |
| White | 30 | 31 | 27 | 24 | 22 |
| Red | 33 | 32 | 34 | 23 | 22 |
| Yellow | 29 | 25 | 21 | 18 | 18 |
| Green | 25 | 23 | 20 | 18 | 15 |
| Grey | 23 | 22 | 25 | 21 | 18 |
| Blue | 55 | | 44 | 41 | 38 |
| White | 56 | | 36 | 42 | 35 |
| Red | 46 | | 39 | 36 | 29 |
| Blue | 57 | | 42 | | |
| Yellow | 56 | | 34 | | |
| Red | 45 | | 35 | | |

We prefer to speak of meridians of the visual field instead of retina, the temporal meridian of the field corresponding to the nasal meridian of the retina, and so on along the circle. The visual field even if it is small extends more in the temporal direction than in the nasal one, and about the same distance up and down. It is thus roughly egg-shaped and resembles greatly the outline of the general field of vision, which some erroneously call the field of vision for white. To compare the extent of the visual field for one color with that for another, we thought it best to determine the average extent in all directions by adding the values obtained for each of the 24 or 12 meridians, and dividing by the number of meridians. The figures for such average meridians are given in table 1. For two subjects, K and L, using 10 mm. discs, the order of extension of the

visual fields is exactly the same and the figures for the individual colors are very close to each other. Blue shows the largest field, and red, white, yellow, green, grey follow in the order named. For the third subject, B, the order for blue, red, white is the same as in the other two, but the field for grey is larger than the fields for yellow and green. With the 5 mm. discs all the fields are smaller. Blue again leads, red and white are smaller and about the same, and grey, green and yellow are smallest, the order being grey, yellow, green. There is then an outer zone where only blue can be made out distinctly, a middle zone where white and red can also be distinguished from the others, and a much smaller region where grey, green and yellow can be individually identified. Each color had to be identified hundreds of times, but although the colored discs were exposed to the eye about the same number of times, there was quite a variation in the mistakes made in naming the different colors.

It is of interest to note the relation between the size of the visual field of a certain color and the ease with which it can be distinguished from the other colors. From tabulation of the mistakes made during the individual experiments, we found that subject K did not once mistake blue for another color; B did that twice. The number of mistakes for blue, which has the largest visual field, was the smallest obtained. Grey follows with no mistake by B and three by K, a total of three. Red comes next with a total of six mistakes, and white follows with a total of twenty. Yellow and green were mistaken the greatest number of times, namely, twenty-three and thirty-seven respectively. With the exception of grey, then, there is a definite correlation between the size of the field and the ease with which the particular color could be correctly identified. For yellow and green, which were mistaken for other colors the greatest number of times, the nature of the mistakes was as follows. Yellow was mistaken for green 11 times and for white 7 times; green was mistaken for yellow 18 times and for white 10 times. White, which was mistaken 20 times, was called grey 17 times. It is evident that the inability to distinguish white from grey was responsible for the tardiness which characterized the naming of this color as it came in from the periphery. Green and yellow were also troublesome, each of them having been mistaken for white a number of times.

It was to be expected that the removal of these confusing colors would give us larger visual fields for white. We performed a group of tests eliminating grey, green and yellow, thus using only blue, red and white. The values obtained are given in table 1, and it will be noted that each of the three visual fields both for 5 and 10 mm. discs were larger than the fields for the same colors when a six color determination was required (fig. 1). The increase in the average meridian for the field of blue was smallest, as would be expected, and it amounted to about 5°. The increase for red

was next in value, equalling 10°; that for white was largest, amounting to approximately 17°. Whereas in practically all cases, with two sizes of discs studied, K's fields of vision were larger than the corresponding fields for B, this difference is accentuated when the number of colors is diminished, and the size of the fields increases. Thus the average meridian for blue, white and red with a 10 mm. disc was 37° for K and 34° for B, when a six color discrimination was required; the same averages, when only the three colors were used, were 52° for K and 40° for B, an increase, on the

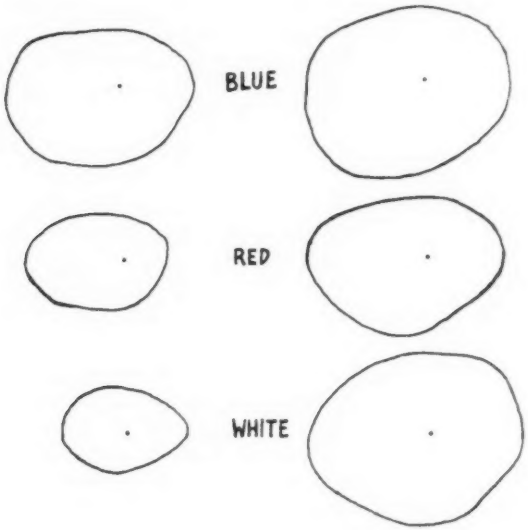


Fig. 1. K, left eye, 10 mm. discs. On the left are the perimeters for blue, red and white, when a six color discrimination test was made. On the right are the perimeters for the corresponding colors, when only blue, red and white were to be discriminated from each other.

average, of 15° for K and 6° for B. When the corresponding averages for 5 mm. discs were obtained, it was found that the increase in the case of K was from 28° to 40°, and in the case of B, from 25° to 35°. The ability to extend the visual field by the diminution in the number of colors to be discriminated is greater in K than in B, but much more so in the case of the larger fields than in the smaller ones.

In another group of tests we again used three colors, blue, yellow and red. As was to be expected the field for yellow was much larger than the one obtained in the first group of tests. Thus, with a 10 mm. disc, and the

the six color discrimination, the average meridian for yellow was 29° for K and 21° for B, with the blue, yellow, red discrimination, the averages were respectively 56° and 34°. We shall discuss the significance of this later.

Before proceeding any further, we decided to find out if there was any difference in the ability of K and B to distinguish colors by means of the fovea, and also whether there was any difference in the size of the fields of general visibility, called by some authors the field for white or grey. For the relative sensitivity of the fovea to color discrimination, we employed 3 and 5 mm. color discs on a black background. The subject approached the colored disc from a great distance looking directly at it all the time. The greatest distance at which he could name the particular color correctly was noted, and duplicate figures were obtained for each of the six colors.

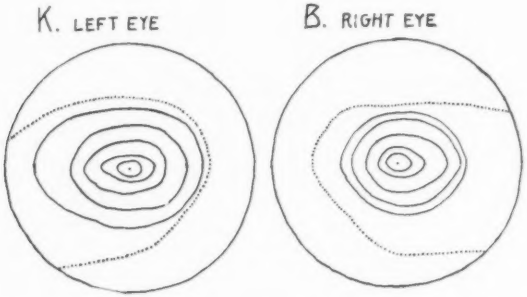


Fig. 2. The visual fields, from inside out, are for figures of 3, 5, 10, 15, 20 mm. having angular sizes of 0.50°, 0.86°, 1.72°, 2.58°, 3.44° respectively. Left eye of K and right eye of B. These perimeters are based on the average values obtained with four geometrical figures: circle, square, triangle, and five-pointed star. Dotted lines indicate general fields of vision of the two eyes.

These figures were about the same for K and B, the distances for some colors being slightly larger in one subject that in the other, and vice versa. Red and white were the most easily seen colors in both subjects, green and yellow follow, and blue and grey were the ones that had to be approached most closely before they could be correctly identified. A few mistakes were made in identification, and consisted in the main of calling yellow white in four cases, grey in two cases, and green in one case.

The general visual fields were determined by moving the black disc from the periphery to the center and asking the subject to report when he first saw "something" in his field of vision. The fields mapped out were normal in both cases, practically following the ones printed on perimeter charts. The average meridian was 58.5° for K and 57° for B, which makes them practically the same (fig. 2).

SECOND SERIES. It having been found that the general fields of vision as well as the color sense of the fovea were identical for both subjects, the differences between the sizes of the visual fields for color could be ascribed only to an unequal number of retinal receptors (cones) in the peripheries of the respective retinas. To test that possibility physiologically we decided to investigate the ability of the peripheral portions of the retina to distinguish geometrical figures, such as a circle, square, triangle and five-pointed star. These figures could be made in a number of different sets, each of uniform dimensions, and the visual fields for each set mapped out.

The literature on peripheral discrimination of form is much smaller than that on color. We could find only two researches that dealt with what is often called visual acuity. One of these represented a study of visual acuity only in horizontal meridians, the temporal and nasal, and was limited to only one subject (Fick, 1898). He found that visual acuity falls off very rapidly at first, slowly afterwards, from the fovea outward in both directions. Discrimination in the periphery is better in the temporal meridian of the visual field than in the nasal one. The other work by Wertheim (1894), done earlier and not so well known, was much more extensive in that four meridians instead of two were explored.

*Method.* For the mapping out of the visual fields for figures we employed the same perimeter that we used previously for colors. The four figures to be distinguished from each other were white on a black background, and were moved in slowly from the periphery until named by the subject. Five sets of figures of different sizes were investigated separately. The circles were 3, 5, 10, 15, 20 mm. in diameter, and the other three figures were inscribed in circles whose diameters were 1 mm. larger, i.e., 4, 6, 11, 16, 21 mm. In this way the differences between the areas of the circles and the other three figures of the particular set were minimized. The angular sizes of the figures of the five sets were 0.50°, 0.86°, 1.72°, 2.58° and 3.44°. Twenty-four meridians were explored with each figure of each set.

*Results.* Twenty separate visual fields were mapped out for each eye studied (left eye of K and right eye of B). The perimeters for the four figures of a given set when superimposed upon each other were found to be practically identical. They crossed and recrossed many times, visibility for one figure along a certain meridian being extended a little farther out than that for another, whereas along the next meridian the situation might be reversed. The average extension for all meridians was obtained in the same way as for color vision, and this average extension was about the same for all figures of a given size. The differences were too small to warrant a separate treatment for the individual figures, and so we determined the average extension of visibility for all figures of each set, for each of the meridians explored. This gave us one perimeter for all the figures of a given size, and the perimeters for the five different sizes are given in

figure 2. It will be noticed at once that the perimeters for the smaller figures, 3, 5 and 10 mm., are identical in both subjects; the perimeter for 15 mm. figures is slightly larger, and for 20 mm. figures much larger in K than in B. The average extension of the visual fields for all meridians studied, for the five sets of figures, were, respectively, 7°, 13°, 24°, 37° and 51° for K, and 7°, 13°, 25°, 35° and 40° for B.

To determine any differences in the ability to recognize the four figures when their images fell on the fovea we employed the same method that we used for colors. Both subjects could distinguish the triangle at the greatest distance while approaching the figures from afar; the circle and the square next; the star had to be looked at closest to be correctly named. In angular sizes these figures had to subtend angles of about 0.075° (4.5′) to be recognized by the foveal retina. This compares very favorably with the 5′ letters of Snellen's test type.

If we consider the visual acuity for any point on the retina to be smaller the greater the angular size of the just distinguishable figure as compared to the size of the figure just seen by the fovea, we can determine the acuity along the lines represented by the perimeters for the figures of various dimensions by dividing the angular size of the particular set of figures by 0.075. If we do this we find that the perimeter for the 3 mm. figure (0.50°) represents a line on the retina where the visual acuity is seven times less than on the fovea (fig. 2). By the same token the other perimeters for 5, 10, 15 and 20 mm. figures pass through zones of the retina where the visual acuity is respectively 15, 23, 34 and 46 times less than that at the fovea.

It is evident that the visual acuity falls off from the fovea outward in both subjects, more rapidly in certain meridians than in others, and in a general way more rapidly in B than in K. We next decided to find out whether this falling off occurred in a definite manner throughout the retina. For this purpose we plotted the extension of visibility along each of the twelve meridians as abscissae with the angular size of the five sets of figures as ordinates (fig. 3). When the abscissa is zero, i.e., when one observes the figure with the fovea, the ordinate is 0.075. It is seen from the figure that the curves obtained for all meridians are straight lines in the case of K, and curved lines, concave upward, in the case of B.

Discussion. The larger fields obtained with the 10 mm. discs as compared to the five would seem to indicate a general thinning-out of the "effective" retinal elements, in this case, the cones. It is generally conceded that there are several million cones and many more million rods, whereas there are only about 500,000 fibers in the optic nerve. It is clear enough that it is not the number of rods or cones in a unit area in one or another portion of the retina that will determine acuity of vision, but the number of fibers in the optic nerve that come from a unit of area in that

region. In the fovea, each cone neurone probably connects through the intermediate neurone with one ganglionic neurone which gives rise to a fiber of the optic nerve. In the periphery of the retina, even though a cone should connect individually with an intermediate neurone, there must perforce be several intermediate neurones to one fiber of the optic nerve. For this reason the effective elements of one or another portion of the retina are the number of ganglionic neurones that make connections with the cones in a unit of area in that region, and it is upon the denseness of these effective elements that the resolving power of the retina depends.

Our results for the four fundamental colors are not in agreement with those of Baird and Engelking in that the fields for blue and yellow and for

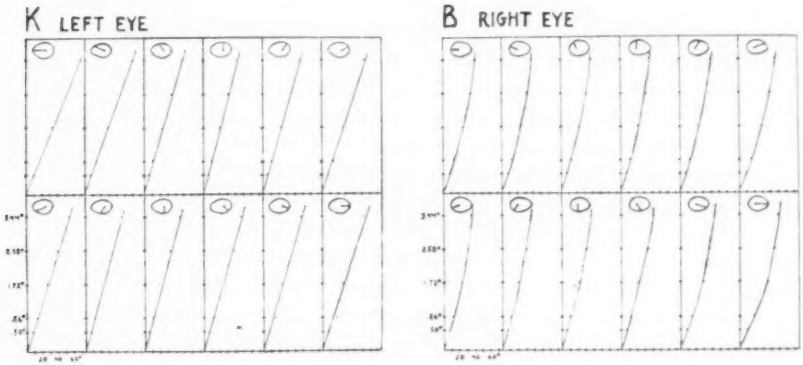


Fig. 3. These curves represent the relationship between the angular size of the figures and the distance from the fovea (in degrees) at which they can just be distinguished from other figures, along each of twelve meridians studied. The particular meridian is indicated in the upper left hand corner of each box. Left eye of K and right eye of B.

red and green are not coextensive. They confirm the data obtained by Aubert and by Peter and others who found that the field for green is always smaller than the field for red. But by introducing grey and white we were able to establish on numerous occasions and in three subjects that the field for white is smaller than that for blue, and that the field for grey, far from being the field for general sensibility for light, is in reality one of the smallest, about the size of the fields for yellow and green. At first our results appeared to us paradoxical. They were clearly against the Hering theory of color vision and in favor of the Young-Helmholtz theory, but this latter assumes that the sensation of white is due to the simultaneous stimulation of the three photosensitive substances, which when individually stimulated give rise to sensations of red, green and blue. However, in

perusing our mistakes, we found that both yellow and green were frequently mistaken for white, and it appeared to us that the smaller field for white was the result of the reluctance on the part of the subject to name white until he could be certain that the disc was neither green nor yellow. To test this possibility we determined the perimeters for red, blue and white only. The field for blue was thereby scarcely affected at all, blue being the most easily recognized color in the periphery of the retina. Some increase in the red was apparently due to the absence of the disturbing yellow and green, but the increase in the field for white was very striking indeed. In K the field for blue and white now became coextensive, but in B that was not quite the case (fig. 1). We were then encouraged to investigate the field for yellow with the disturbing white and green absent. In mapping out the fields for blue, yellow and red, we obtained results that resembled very strikingly indeed the figures for the blue, white, red discrimination tests. The average meridian for the field of vision for yellow increased from 29° to 56° in the case of K and from 21° to 34° in the case of B. This partially explains why some investigators who did not use white found blue and yellow to be coextensive.

It seems then that not only are the retinal fields for the various colors different, but their size depends upon the number and kind of colors that the subject is required to distinguish from one another. Since one cannot suppose that the effect made upon the retinal elements by a red, blue or yellow disc of a certain size is dependent upon the number of colors the subject is called upon to discriminate, we must conclude that there is a central (cerebral) factor which plays an important part in determining the size of the visual fields. The fewer the number of colors (especially confusing colors) the surer the subject feels of his recognition of a particular color.

Our results also point to the erroneousness of designating the field of general sensibility to light as the field for either white or grey. Looked upon as a color, grey has a very small visual field, and for the following reasons. Most colored discs on a black background appear as bright spots when they fall on the periphery of the retina, as was already pointed out by Aubert. As the grey spot moves in, the subject reserves his judgment until he is sure that the bright spot is not going to appear blue, red, or any other color, and only then is he prepared to call it grey on the basis of its luminosity.

Insofar as the thinning out of the effective retinal elements is concerned this does not take place in all meridians at the same rate, but proceeds more rapidly on the temporal half of the retina (nasal half of visual field) than on the nasal one. Moreover, there is an individual variation in the rate at which the thinning proceeds along any given meridian. Thus, when one compares the visual fields for the different colors using the 10 mm.

discs one can see that the thinning took place at about the same rate in the eyes of K and L, at a greater rate in B (table 1). This came especially in evidence when only three colors were used and large visual fields were obtained. The visual field for the blue in K extended on an average to 56° from the fovea, but only to 43° in B. With the 5 mm. discs, the differences in the sizes of the visual fields for colors were practically negligible.

It is evident that in the various meridians from the fovea outward, the thinning of the effective retinal elements goes on at about the same rate in both K and B for about 20° on the average, but farther out the decrease in B becomes more marked as compared to K so that the density of the retinal elements in the forties of B is about the same as that in the fifties of K. It is to test the correctness of this supposition that we undertook the mapping out of the visual fields of geometrical figures of five different sizes. As was seen from figure 2, the results fully confirmed our expectations. Furthermore, in figure 3 we demonstrated that the thinning occurs in the same manner in all meridians of the retina of a given eye, the curve being a straight line for every meridian of K's eye and a curve concave upward in every meridian of B's eye. Two cases are insufficient for generalizations, but the constancy of the nature of the curve for all the meridians of each of the two eyes studied would seem to point to the manner of thinning out of the retinal elements as characteristic of a given retina throughout its extent.

We quite agree with Wertheim that the maximum extent of the visual field can be obtained only after a long practice. But not only the extent of the visual field but also the smoothness of its outline shows a great improvement with exercise. It is doubtful if any information can be obtained by examining the periphery of a patient's retina *once* for the discrimination of either color or form.

We thank Dr. A. B. Luckhardt, who served as a subject in some tests and who gave us some valuable suggestions in the course of this work.

## SUMMARY

1. The visual fields as determined simultaneously for six colors in three subjects can be arranged as follows in decreasing order in extension: blue, red, white, yellow-green-grey.

2. A central or cerebral element affecting the extent of the visual field was demonstrated. The fields for blue, red, white and yellow will be larger, when the number of colors to be discriminated is smaller. That applies especially to yellow and white.

3. There is an individual variation in the extension of the fields of vision for different colors.

4. The fields of vision for form as determined by discrimination of geometrical figures are practically identical for figures of the same size, and are larger the greater the size of the figure.

5. Visual acuity, both for color and form, falls off from the fovea outward, but at an unequal rate in different directions.

6. The manner in which the visual acuity decreases is the same along all meridians explored and would seem to be a personal characteristc (based on two subjects).

7. The ability to distinguish color and form in the periphery of the retina improves with practice.

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# OBSERVATIONS ON THE INTRACAPSULAR PRESSURE AND THE MOLECULAR CONCENTRATION OF THE RENAL CAPSULAR FLUID IN NECTURUS

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The view that glomerular function is a process of filtration may be said to be almost universally accepted by physiologists today. A great mass of presumptive evidence favoring the view of glomerular filtration in the mammalian kidney has accumulated. The nearest approach to direct evidence on this point has been made with the frog. Hayman (1927) found that in 141 of 181 estimations the glomerular capillary pressure in the frog lay between 10 and 27 cm. of water, in 29 the pressure ranged from 27 to 52 cm. of water. In only 11, or 6 per cent of the estimations, was the pressure lower than 10 cm. of water, the average figure found by White (1924) for the colloidal osmotic pressure of frog's plasma. It would thus seem that in the frog the glomerular capillary pressure is high enough to overcome the colloidal osmotic pressure of the plasma provided we assume that the plasma colloidal osmotic pressure of Hayman's frogs was not too much higher than that of White's frogs. Filtration will now occur, provided that the pressure in the lumen of the capsule is not too high. This last possibility does not seem to have received in recent times the attention which it merits; the fact is sometimes overlooked that only the difference between glomerular capillary pressure and intracapsular pressure is the effective head of filtering pressure. It has usually been assumed in recent publications that intracapsular pressure is not significantly higher than atmospheric; any one who has seen the distended capsules of an actively functioning frog or Necturus kidney would hardly, however, be willing to grant this assumption without proof. One might infer from Hayman's statement, "A slight intracapsular pressure was sufficient to distend Bowman's capsule," that the assumption of a negligible intracapsular pressure is correct; figures on the amount of pressure necessary to effect the first entrance of the dye into the capsule are not given.

The arguments of Tamura and collaborators (1927) in favor of a normally high intracapsular pressure in the frog are quite unintelligible to me. They state that the pressure in a manometer attached to the ureter may rise to

12 or 15 mm. Hg and that when it is raised to 20 mm. Hg by injection of salt solution into the rubber tubing joining the manometer to the ureter, glomerular flow ceases. This is, of course, what would be expected from a kidney with a glomerular capillary pressure which can be raised by obstruction to a level somewhat below 20 mm. Hg, but how any conclusions can be drawn as to the pressure normally obtaining in the capsular lumen with the ureter unobstructed is far from clear. Their statement, "Therefore, it is clear that the glomerular capillary tuft always lies under quite a high extra-capillary pressure," is quite without foundation. These investigators' failure to prove a high intracapsular pressure does not, however, prove that such pressure does not exist.

In the first part of this paper are given the results of determinations of the intracapsular pressure, with the tubule unobstructed, in Necturus. It was found that this pressure varied from 1 or 2 up to 57 mm. water in different capsules. The determinations are carried out very simply. A leveling bulb is connected to the micropipette by rubber tubing and the system filled with water. A little saturated solution of trypan blue is sucked into the tip of the pipette and the level of water in the bulb brought into the same horizontal plane as the tip of the pipette. The pipette is then inserted into the capsule of a glomerulus in active circulation and the leveling bulb raised until the first movement of dye out of the pipette is seen. The height of leveling bulb at which this occurs is very sharply defined, a change of level of less than 1 mm. bringing about an easily recognizable reversal of flow of the dye. Controls show that a head of pressure of less than 1 mm. is sufficient to start a flow of dye from the pipette into a dish of water into which the pipette dips. Thus, with the tip of the pipette 2 mm. below the surface of water in an 11 cm. Petri dish, the first movement of dye out of the pipette occurred when the level of water in the leveling bulb was between 3 and 4 mm. above the tip of the pipette. Two millimeters represents the back pressure of the column of water above the pipette in the dish. Furthermore, except for this back pressure, the tip of the pipette is under atmospheric pressure when the water level of the leveling bulb is between 1 and 2 mm. above the pipette tip, since this is the height of the water column supported by the capillarity of a tube of 15 mm. bore, that of the leveling bulb. It is thus seen that theoretically an infinitesimal head of pressure will move the dye out of the pipette and this condition is realized in practise. Hayman's finding that a head of pressure up to 16 mm. water may be required to bring about the first movement of the dye out of the pipette is almost certainly due to a deformation of the rubber membrane of his capsule produced by the insertion of the pipette, this deformation producing an increased pressure in the capsule which apparently was not taken into account. It may be of interest to mention that if the tip of the pipette is inserted into a column of water in a hori-

zontal capillary tube, the dye begins to leave the pipette when the level of water in the leveling bulb is below that of the tip of the pipette by a distance equal to the length of the column of water which that tube will support by capillarity. This affords a rather amusing demonstration of the negative pressure inside a column of liquid in a capillary tube. Protocols of intracapsular pressure determinations on 11 capsules in 3 necturi are given.

*Experiment 1.* March 2. Male. Circulation only fair, many glomeruli show no circulation. First capsule entered had relatively small amount of fluid, capillary flow was steady but slow. Dye entered when level in bulb was between 2 and 3 mm. above tip, i.e., about 1 mm. effective head of pressure. By lowering bulb less than 1 mm. from the equilibrium level a slow flow of capsular fluid was seen to enter the pipette; when the bulb was raised the dye entering the capsule was seen to be slowly washed down the tubule. These observations prove that the glomerulus was functioning even though not briskly. Two other capsules showed the same findings as the first. A fourth capsule, in the other kidney, showed a pressure of 4 mm. in its lumen: it was appreciably more distended and the glomerular circulation was perhaps more active.

*Experiment 2.* March 3. Male. Circulation excellent, capsules exhibiting the most active circulation were well distended. No evidence of alternation of phases of activity of individual glomeruli, i.e., those sluggish remained sluggish and had only poorly filled capsules. First capsule, dye entered at 21 mm., reversal point very sharply defined, less than 1 mm. Second capsule, dye entered at 45 mm. The intracapsular pressure varied between 42 and 47 mm. for a period of 15 minutes, then dropped to 30 mm. for about a minute, returning to the 42 to 47 mm. level and remaining there for another 20 minutes, as long as it was followed. Third capsule, pressure in lumen stayed at 33 to 36 mm. water for 5 minutes. It was quite obvious that the rate of passage of water through the glomerular membrane was much higher in this animal than in the first animal. On a slight lowering of the bulb the capsular fluid entered the pipette more rapidly and dye injected into the capsule was washed into the tubule more rapidly. If enough dye to color the entire capsular contents was injected it was cleared out in from 30 to 60 seconds.

*Experiment 3.* March 3. Female. Circulation quite active in many capsules, well distended, no alternation seen. First capsule, intracapsular pressure between 29 and 32 mm. water for 15 minutes. Second capsule, the circulation strikingly active. Intracapsular pressure 57 mm. water, varied between this and 50 mm. for 10 minutes, slowly fell to 40 mm. during next 15 minutes, not followed any longer. Third capsule, intracapsular pressure between 34 and 37 mm. for 20 minutes. Fourth capsule, pressure between 15 and 18 mm. for 5 minutes. Circulation appeared active but capsule was perhaps less distended than first three.

It is quite apparent that the capsules with the most active glomerular circulation show the highest pressure, while a feeble glomerular circulation is accompanied by and presumably stands in causal relationship to a low intracapsular pressure. The most plausible explanation of this relationship and one for which evidence is seen in the foregoing protocols is that the elimination of water by the glomeruli exhibiting a brisk circulation is more rapid, the intracapsular pressure rising until the rate of escape of glomerular fluid down the tubule equals its rate of formation. It is of course conceiv-

able that changes in intracapsular pressure might be brought about, the rate of glomerular fluid elimination remaining constant, by active changes in the caliber of some part of the tubular system, but there is no convincing evidence of any such sphincter-like action. An observation which has frequently been made and which at first thought might seem to favor the idea of a tubular sphincter is that of a kidney with generally poor circulation, where many capsules are well distended with fluid at a time when all circulation through their glomerular capillaries has ceased. We believe, however, that this tubular blocking is a manifestation of injury rather than a physiological phenomenon.

The results reported here would seem to make it worth while to continue this type of investigation. We propose to carry out in one and the same animal simultaneous (or as nearly so as is possible) determinations of the glomerular capillary pressure, intracapsular pressure, the colloidal osmotic pressure of the plasma and the rate of urine flow. Such determinations will tell us first, whether the effective head of pressure, i.e., the difference between glomerular capillary pressure and intracapsular pressure, in an active glomerulus exceeds the plasma colloidal osmotic pressure; second, whether any relation between the height of this effective head of pressure and the rate of urine flow can be established, and third, whether it is possible for any glomerular elimination of water to continue (by some process other than simple filtration) at a time when the effective head of pressure is less than the colloidal osmotic pressure of the plasma.

The second part of the present paper deals with a different topic, the molecular concentration of the glomerular fluid. It has long been assumed that the glomerular fluid is simply deproteinized plasma. This view was not incompatible with the qualitative findings of Wearn and Richards (1924) on the frog and of White and Schmitt (1926) on Necturus. The first quantitative determinations on glomerular fluid were those by Wearn and Richards (1925) on chlorides. They found a higher chloride concentration in glomerular fluid than in plasma and concluded that, "The results may serve as the basis of a reasonable doubt of the complete adequacy of the present conception of glomerular filtration." That opportunities for error in such work are numerous and easy has, of course, been appreciated by these authors. The only other paper presenting quantitative data on glomerular fluid is that of Schmitt and White (1928) who showed that its inorganic phosphate content is only $\frac{1}{4}$ to $\frac{1}{3}$ that of the plasma. This finding is explained on the basis that only this fraction of the inorganic phosphate is present in a filterable state; it presents no difficulties to the theory of simple filtration.

The writer was impressed with the magnitude of the difficulties inherent in and the elaborateness of the precautions essential to the analytical procedure employed by Wearn and Richards. The conviction persisted that,

in spite of Wearn and Richards' failure with the freezing point method, a determination of the molecular concentration of the glomerular fluid offered the greatest hope of obtaining convincing results. The method described by Barger (1904) based upon vapor tension and applicable to very small amounts of fluid was therefore investigated. This method depends upon the fact that if a column of a solution of a higher molecular concentration is placed between two columns of a solution of lower molecular concentration in a sealed capillary tube, the column of stronger solution will increase in length at the expense of the columns of weaker solution, the process continuing until the solutions in all the columns are of equimolecular concentration. This is because the vapor tension of the weaker solution is higher than that of the stronger; the solvent consequently evaporates from the weaker into the stronger. The lengths of the columns are read under the microscope soon after the tube is prepared and again after standing for about 24 hours. Numerous controls were carried out to establish the validity of the method as applied to a comparison of blood serum and glomerular fluid. One disconcerting source of the irregular results seen in many of the tables in Barger's paper and recognized by him is the variation in temperature of the contents of the tube during their equilibration. It has been possible in the present work to escape this irregularity by keeping the tubes throughout the period immersed in a Petri dish of water in a warm room at a constant temperature of 34.5°C. The water was poured into the dish in the warm room from a large bottle which was kept in the room. The temperature was controlled by an electric thermostat and did not vary more than 0.5°C. Under these conditions there was practically no change in the length of neighboring columns of the same solution, i.e., there was usually no change and never one greater than three-tenths of a division on the micrometer scale.

A difference in concentration of 0.05 per cent NaCl, for instance 0.60 per cent as compared with 0.65 per cent NaCl, is the smallest with which the method as used will show consistently significant changes. The method is capable of detecting smaller differences if larger tubes are used; my reason for using small tubes (800$\mu$ inside diameter) will be referred to later.

Since it is quite possible that the surface tension of the serum is different from that of the glomerular fluid, controls were carried out to see whether errors due to a "creeping effect" of the solutions along the capillary wall are produced when solutions of different surface tension but the same osmotic pressure are compared. A solution of 0.7 per cent NaCl was compared with the same plus 10 mgm. sodium taurocholate per 100 cc.; the minute change in molecular concentration brought about by the addition of this amount of bile salt is not demonstrable by this method. The surface tension of the pure NaCl solution determined on the du Noüy

ring instrument was 74.4 dynes per cm., of the NaCl plus sodium taurocholate 54.7 dynes per cm. We have followed Barger's practise of having a relatively long column, which was not measured, at each end of the series with 5 shorter columns between. Thus, in the experiments described in this paragraph a long column of NaCl solution was drawn into the tube, then a short column of NaCl plus sodium taurocholate, this being the first one measured, then NaCl, NaCl plus sodium taurocholate, NaCl, NaCl plus sodium taurocholate, this being the last one measured, then a long column of NaCl; an air column 1 to 2 mm. long separated the neighboring liquid columns.

Two such tubes were prepared; the results are seen in table 1. Lengths of columns are given in scale units, one scale unit being 40$\mu$ with the lens system used. It is apparent that the lengths of the columns remain unchanged; liquids of different surface tensions can therefore be safely compared.

TABLE 1

*Lengths of columns in scale units*

| DATE | 0.7 PER CENT NaCl PLUS BILE SALT | 0.7 PER CENT NaCl | 0.7 PER CENT NaCl PLUS BILE SALT | 0.7 PER CENT NaCl | 0.7 PER CENT NaCl PLUS BILE SALT |
|---|---|---|---|---|---|
| Tube 1: | | | | | |
| February 2, 4 p.m. | 33.2 | 46.8 | 78.5 | 38.6 | 19.7 |
| February 3, 10 a.m. | 33.2 | 47.0 | 78.4 | 38.8 | 19.4 |
| February 3. 4 p.m. | 33.4 | 47.1 | 78.5 | 38.8 | 19.6 |
| Tube 2: | | | | | |
| February 2, 4 p.m. | 27.1 | 34.9 | 28.7 | 32.8 | 22.9 |
| February 3, 10 a.m. | 27.3 | 34.9 | 28.4 | 32.8 | 22.9 |
| February 3, 4 p.m. | 27.3 | 34.9 | 28.4 | 32.8 | 22.9 |

Similar controls were carried out with human serum and cat serum. The freezing point of a serum was determined by a thermocouple method. A NaCl solution of the same freezing point was then made up; the data for making up NaCl solutions of known freezing points were obtained from Landolt-Börnstein tables and checked with a Heidenhain thermometer. Tubes were then prepared containing alternating columns of serum and of NaCl solution of the same freezing point; in some cases a single column of salt solution was between two colums of serum, with a longer column of serum at each end, since this was the arrangement used with glomerular fluid. In no case was there a significant change in the lengths of the columns when serum was thus compared with a salt solution of equimolecular concentration. To establish further the validity of the method for comparing the concentrations of protein-containing and protein-free

solutions some cat's blood was allowed to clot and centrifuged for 2 hours in an open tube. Considerable evaporation took place, the serum having a $\Delta$ of 0.74°C. which is equivalent to 1.27 per cent NaCl. A series of tubes, each containing alternating columns of serum and standard NaCl solution was then prepared. The first tube contained serum and 1.1 per cent NaCl, the second serum and 1.2 per cent NaCl, the third serum and 1.25 per cent NaCl, the fourth serum and 1.30 per cent NaCl, the fifth serum and 1.35 per cent NaCl and the sixth serum and 1.40 per cent NaCl. In the first three tubes the serum columns increased and the salt columns decreased in length, the extent of change decreasing from the first to the third; in the last three tubes the serum columns decreased and the salt columns increased in length, the extent of change increasing from the fourth to the sixth. It is thus seen that not only do the columns fail to change in length when serum is compared with the salt solution of the same concentration, but a reversal point is seen when it is compared with solutions of lower and higher concentrations.

We next turned to a comparison of glomerular fluid with the serum of the animal obtained immediately after the collection of the fluid. The theory of the procedure is simple enough; collect glomerular fluid, place it between two columns of serum and see whether or not it changes length. It may be said here that, somewhat to our surprise, in every case the column of glomerular fluid did become longer. Some remarks on the technical procedures and possibilities of error will precede the presentation of data. The glass micropipettes had an outside diameter at the tip of 10 to 16 $\mu$, and an inside diameter of about 7 to 12$\mu$. The pipette was held in a Chambers micromanipulator and connected to a leveling bulb by a rubber tube and glass adaptor. The bulb, tubing and pipette were filled with mercury; positive or negative pressure was applied by raising or lowering the bulb on a Zimmermann stativ. No anesthetic was used, the brain being pithed. This plan had been tried long before and abandoned, as the circulation was usually greatly depressed. The success in the present series of experiments depends upon the fact that the needle was inserted between the skull and spinal column while the animal was free in the water. If one holds an animal while pithing it, as is done with a frog, it struggles violently and in only a low percentage of the cases is the circulation satisfactory; presumably the injury to the gills inflicted by the experimenter's hand is what causes the damage. Pithing the animal "on the run" demands a certain degree of dexterity but when it is nicely executed it affords the most satisfactory preparations. The animal is fastened on the special board with its head in water, a kidney exposed and, under the microscope, the pipette inserted into the capsule of an actively functioning glomerulus. The bulb is lowered until the fluid begins to enter the pipette. This happens when the level of mercury in the bulb is appreciably above the

tip of the pipette, due to the positive intracapsular pressure and the surface tension of mercury. The attempt is made to keep the bulb at such a level that the capsule wall is just not collapsed, so that there is no strong suction. If the level of the mercury in the bulb is a trifle low the capsule wall collapses around the pipette; in this event the bulb is raised (a fraction of a millimeter may suffice) until the fluid again just lifts the capsule wall. I have felt that it is better to let some of the fluid escape down the tubule than to create a strong suction. The fluid rises steadily in the pipette and in from 5 to 12 minutes enough has accumulated (0.5 to 1 cu. mm.) for a test. After the first three experiments it was recognized that the objection might be raised that the higher molecular concentration of the glomerular fluid might be due either to an evaporation of water through the capsule wall or to a leakage into the capsule and thus into the pipette of concentrated (through evaporation) body fluid through the spot where the pipette tip pierced the capsule wall. Although there is abundant evidence that the seal where the pipette pierces the wall is perfectly tight it was thought best to test the validity of both these objections by keeping in all subsequent experiments frog Ringer's diluted to one-half strength with distilled water constantly playing over the region of the kidney containing the capsule under observation. One drop every 3 or 4 seconds fell from a height of 2 or 3 mm. about 5 to 8 mm. from the capsule, keeping the surface of the capsule flooded. This was, of course, not started until just after the pipette was inserted. In this way any possibility of evaporation of water through the capsule wall was prevented; furthermore, if there had been any leakage of outside fluid into the pipette it would have made the fluid in the pipette weaker instead of stronger. It may also be pointed out that this arrangement rules out the possibility that the higher molecular concentration of the capsular fluid might have been due to the entrance of concentrated body cavity fluid into the capsule by way of the nephrostome.

After sufficient fluid had accumulated in the pipette the leveling bulb was raised a millimeter or so and the pipette immedately withdrawn and its tip washed with distilled water from a wash bottle. There can be no appreciable evaporation through the tip even for a very much longer time than was required to collect and centrifuge the blood and transfer the glomerular fluid into the receiving capillary. Immediately after the pipette was withdrawn and washed, the animal's aorta was exposed and blood drawn; in the first and ninth experiments blood was taken from the abdominal vena cava instead of the aorta. In the first five experiments the blood was drawn into a glass bulb drawn out at one end into a tip of 150–200$\mu$ diameter which was inserted into the aorta, and at the other end into a tube 2 or 3 mm. in diameter, over which was slipped a rubber tube. After the tip had entered the blood vessel the blood was sucked up, suction being applied by mouth through the rubber tube. The tubes on each end

of the bulb were emptied of blood and sealed in a fine flame and the contents then centrifuged. After the first five experiments it was recognized that the objection might be raised that a loss of $CO_2$ during the drawing of the blood had lowered its molecular concentration so that the higher concentration of the glomerular fluid was not necessarily a normal condition. In order to test the validity of this objection blood in the subsequent experiments was drawn without loss of $CO_2$ by using a bulb such as is shown in the illustration (fig. 1). The tip, *A*, was inserted into the aorta and the blood sucked up to *B* by suction applied at *C*. The tube was then broken at *D* and the blood to the left of *D* discarded. This was enough to form a column 1.5 to 2 cm. deep even in the wide part of the bulb, i.e., the retained blood had always been separated from the air by a layer of blood that deep. The tubes at each end of the bulb were now emptied of blood for about a centimeter from their ends by inserting a smaller capillary, the ends sealed and the blood centrifuged.

The next step was to transfer the serum and glomerular fluid to the receiving capillary. A word may be said here about the choice of size of

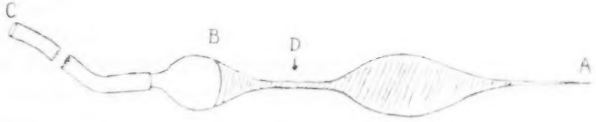


Fig. 1

capillaries. It had been learned from past experience that from 0.5 to 1 cu. mm. of fluid could be collected from an active capsule in about 5 to 10 or 12 minutes. It seemed desirable to keep the collection period as short as possible in order that there be no time for the composition of the blood to change between glomerular fluid and blood collections. This had been recognized by Wearn and Richards (1925) as a possible source of serious error. The fact that the collection period in my experiments is a matter of minutes instead of hours is, I believe, a great advantage. It is true that Wearn and Richards in their last three experiments used a composite sample of blood made up of several small samples taken at intervals throughout the period of glomerular fluid collections and in two of these still found a significantly higher chloride concentration of the glomerular fluid than of the serum; it will hardly be denied, however, that an interval of from 4 to 8 minutes between the middle of glomerular fluid collection period and the time of taking the blood sample affords a more certain basis of comparison. Having thus limited our collection time, our glomerular fluid sample was limited to a maximum of 1 cu. mm. It was

then necessary to determine by trials with water or salt solutions (before the series of animal experiments was undertaken) the safe maximum size of capillary receiving tube into which this volume of liquid could be inserted. If too large a tube is used the fluid does not form a column occupying the entire cross section of the tube but simply runs along the wall. It was found that a tube of 800$\mu$ inside diameter was the largest that could be used and this size was therefore employed in all the experiments. As Barger has pointed out, the sensitivity of the method is decreased as the diameter of the tubes decreases; he used tubes of 1.5 mm. inside diameter. For the present purpose, however, high sensitivity is of no importance; in fact, the demonstration of difference in molecular concentrations of the fluids compared becomes more convincing when it is remembered that the observed changes in length were obtained in tubes which keep the sensitivity of the method much below its maximum.

In the first three experiments the procedure of preparing the tubes was as follows. A column of serum 2 to 4 mm. long was drawn into the tube by capillarity, this was then drawn back about 2 mm. from the end either by gravity or by applying careful suction to the other end of the tube by mouth. The tip of the micropipette was then quickly inserted into the capillary and the glomerular fluid expelled by raising the leveling bulb. In three or four of the experiments it was necessary to break off the extreme tip of the pipette in order to effect the expulsion of fluid. This was due to slight traces of protein-containing body fluid which had adhered to the tip as it was being withdrawn from the capsule, which had failed to be washed off and formed a slight film over the tip on drying.

It may be worth while here to give a brief calculation to prove that there is no possibility of the molecular concentration of the fluid in the pipette having been raised either by evaporation through the tip while the blood was being collected and centrifuged or by the entrance of concentrated body fluid into the pipette as it was being withdrawn or inserted. First, as to evaporation from the pipette. Ten $\mu$ may be taken as a high figure for the inside tip diameter of the average pipette; most of them were smaller than this. This means an exposed area at the tip of about 80 sq. $\mu$. The volume of the sample may be taken as 0.5 cu. mm. or 500,000,000 cu. $\mu$. Therefore, the extent to which the fluid in the pipette could have been concentrated by evaporation during the 15 minutes that it stood during the collection and centrifuging of the blood is the same as that to which a volume of 1 cc. of solution could be concentrated in 15 minutes by evaporation from an area of about 0.0016 sq. cm., which is entirely negligible. Next, as to the possibility of contamination by entrance of concentrated body fluid on inserting the pipette. The average cross section area of the pipette for a distance of 20 $\mu$ back from the tip is about 10 sq. $\mu$ and, by having the leveling bulb raised about 40 cm., the mercury column comes to

within 20 $\mu$ of the tip while the pipette is being inserted. Now, it is quite easy to see that this air-filled space is never filled with fluid until the leveling bulb is lowered, but even if it became completely filled with 10 per cent NaCl solution while the pipette tip was penetrating the capsule wall this would be a volume of only 2,000 cu. $\mu$, which in a volume of 500,000,000 cu. $\mu$ could increase the concentration of the fluid by only 0.00004 per cent NaCl. There can be no contamination by body fluid while the pipette is being removed from the capsule because there is now, of course, fluid out to the very tip of the pipette and it is under a mm. Hg or so positive pressure; furthermore, the pipette is clear of the kidney in a fraction of a second, the animal board being jerked away from the pipette rather than the pipette being slowly removed from the capsule.

As soon as the glomerular fluid had run into the receiving capillary this was dipped again into serum and another column (in the first three experiments) 2 to 4 mm. long drawn up. Only a few seconds elapse from the time the glomerular fluid enters the tube until the tube is dipped into the serum. It is quite inconceivable that any significant concentration of fluid by evaporation could take place in this time. The columns are next gravitated or sucked back until the end of the serum column is about 1 cm. from the end of the receiving tube and both ends are then sealed in a fine flame. The tube is fastened to the bottom of a Petri dish by plasticine and the dish and microscope taken into the warm room. Water from a large bottle kept standing in the warm room is poured into the dish, which is then placed on the stage of the microscope and allowed to stand for 3 to 5 minutes, when the length of the glomerular fluid column is read. Another reading is then made in about 24 hours, sometimes additional readings at other intervals were made.

After the first three experiments it was recognized that in order to be sure that the increase in length of the glomerular fluid column was not due to the chance operation of some unknown factors, it would be desirable and quite practicable to have a short and measurable column of serum on each side of the column of glomerular fluid, in addition to a long "buffer" column of serum, which was not measured, on each extreme. If the increase in length of the glomerular column is really due to a higher molecular concentration we should then also see a decrease in length of the serum column on either side of it. The arrangement will be clear from figure 2. This arrangement was therefore carried out in the last nine experiments, and in every case there was a definite decrease in the serum columns as well as an increase in the glomerular column. The meniscus outline stands out very sharply and it is easily possible to read the length of a column to $\pm$ 0.1 scale unit.

Although it had been demonstrated that the vapor tension method gave the same values in determinations of the molecular concentration of salt

solutions containing bile salts and of cat and human serum as did the freezing point method it seemed that the experiments would be more convincing if it could be shown that the same values of molecular concentration of Necturus serum could be obtained with the vapor tension and freezing point methods. For if this were demonstrated it would remove any doubt that might exist as to the justifiability of interpreting the changes in length of the columns as being due to differences in molecular concentration and to these differences alone. Therefore, in the last four experiments, in addition to comparing the glomerular fluid with the serum, the absolute value of the molecular concentration of the serum was determined by both the vapor tension and freezing point methods. The two methods agree; therefore it can be stated with certainty that the increase in length of glomerular fluid column and decrease of serum columns are due to a higher concentration of the former.

Table 2 gives the results of the comparison of glomerular fluid and serum in the twelve experiments. Table 3 gives the complete data of experiment 9, a typical experiment of the last four, where the absolute value of the molecular concentration of the serum was obtained both by the freezing

Fig. 2

point method and by the vapor tension method, using a series of tubes containing standard NaCl solutions of different strengths for comparison. It will be noted that in every case in table 2 the glomerular column increased in length and the serum columns (except in the first three experiments, where these were not measured) decreased. It may be pointed out that one should not expect the sum of the decreases in length of the two serum columns to equal exactly the increase in length of the glomerular fluid column, for the loss of water from the adjoining serum columns into the glomerular fluid column will be partly made up by the evaporation of water from the long serum columns at the extremes of the series. It will be seen in table 2 that the increase in the length of the glomerular fluid column is greater than the sum of the decreases of the two measured serum columns, and this is to be expected. It is impossible to state just what is the degree of difference of concentration between glomerular fluid and serum, but from a study of the data of the last four experiments it can be said that the change in length is usually about the same as that seen when a NaCl solution 30 to 50 millimols stronger than the serum is compared with it, i.e., if the serum is equivalent to 0.50 to 0.55 per cent NaCl the glomerular fluid is the equivalent of 0.60 to 0.70 per cent NaCl. In two

TABLE 2

*Lengths of columns in scale units*

| NUMBER | DATE | SERUM | GLOMERULAR FLUID | SERUM |
|---|---|---|---|---|
| 1 | January 18, 4 p.m. | | 22.4 | |
| | January 19, 10 a.m. | | 24.2 | |
| | January 19, 5 p.m. | | 24.4 | |
| | January 20, 9 a.m. | | 24.7 | |
| | January 23, 9 a.m. | | 25.2 | |
| 2 | January 19, 12 m. | | 16.7 | |
| | January 19, 5 p.m. | | 17.1 | |
| | January 20, 9 a.m. | | 18.3 | |
| 3 | January 20, 3 p.m. | | 50.7 | |
| | January 21, 10 a.m. | | 51.4 | |
| | January 23, 10 a.m. | | 53.2 | |
| 4 | January 26, 4 p.m. | 24.2 | 26.3 | 64.3 |
| | January 27, 10 a.m. | 23.8 | 27.4 | 63.8 |
| | January 28, 9 a.m. | 23.6 | 28.6 | 63.0 |
| 5 | January 28, 11 a.m. | 40.9 | 15.3 | 30.9 |
| | January 28, 4 p.m. | 40.6 | 15.8 | 30.4 |
| | January 30, 10 a.m. | 39.8 | 17.7 | 29.8 |
| 6 | January 30, 1 p.m. | 59.5 | 24.9 | 14.4 |
| | January 30, 4 p.m. | 59.2 | 25.6 | 14.2 |
| | January 31, 11 a.m. | 59.0 | 26.2 | 14.0 |
| 7 | January 31, 4 p.m. | 43.9 | 31.8 | 27.8 |
| | February 1, 1 p.m. | 43.7 | 32.9 | 27.1 |
| | February 2, p.m. | 43.5 | 34.7 | 26.8 |
| 8 | February 16, 4 p.m. | 13.0 | 15.2 | 12.9 |
| | February 17, 10 a.m. | 12.6 | 16.2 | 12.5 |
| | February 18, 10 a.m. | 12.2 | 16.9 | 11.8 |
| 9 | February 17, 5 p.m. | 32.0 | 23.7 | 28.4 |
| | February 18, 11 a.m. | 31.4 | 24.9 | 28.0 |
| | February 18, 5 p.m. | 31.1 | 25.2 | 27.8 |
| 10 | February 22, 3 p.m. | 17.5 | 32.0 | 19.7 |
| | February 23, 11 a.m. | 17.2 | 33.0 | 19.4 |
| 11 | February 23, 3 p.m. | 28.9 | 35.4 | 10.2 |
| | February 24, 11 a.m. | 28.0 | 36.8 | 9.9 |
| 12 | February 24, 11 a.m. | 24.3 | 28.4 | 17.2 |
| | February 25, 10 a.m. | 23.9 | 29.9 | 16.5 |

or three experiments the changes in length were even greater than would correspond to these differences in molecular concentration.

In order to determine the freezing point of Necturus serum it was necessary to use a method applicable to small amounts. I have used a thermocouple constructed and described by Bishop (1923). With the

TABLE 3

| DATE | SERUM | GLOMERULAR FLUID | SERUM | | |
|---|---|---|---|---|---|
| February 17, 5 p.m. | 32.0 | 23.7 | 28.4 | | |
| February 18, 11 a.m. | 31.4 | 24.9 | 28.0 | | |
| February 18, 5 p.m. | 31.1 | 25.2 | 27.8 | | |
| | Serum | 0.7% NaCl | Serum | 0.7% NaCl | Serum |
| February 17, 5 p.m. | 25.1 | 36.9 | 35.0 | 37.8 | 28.8 |
| February 18, 11 a.m. | 24.0 | 38.0 | 34.0 | 39.0 | 27.1 |
| February 18, 5 p.m. | 23.8 | 38.4 | 34.0 | 39.2 | 27.0 |
| | Serum | 0.6% NaCl | Serum | 0.6% NaCl | Serum |
| February 17, 5 p.m. | 26.5 | 38.4 | 31.6 | 29.4 | 26.0 |
| February 18, 11 a.m. | 26.1 | 39.0 | 30.9 | 29.9 | 25.8 |
| February 18, 5 p.m. | 26.0 | 39.1 | 30.7 | 30.0 | 25.7 |
| | Serum | 0.5% NaCl | Serum | 0.5% NaCl | Serum |
| February 17, 5 p.m. | 15.2 | 42.8 | 12.6 | 40.5 | 14.8 |
| February 18, 11 a.m. | 15.6 | 42.5 | 12.7 | 40.2 | 14.8 |
| February 18, 5 p.m. | 15.8 | 42.4 | 12.7 | 40.0 | 14.8 |
| | Serum | 0.4% NaCl | Serum | 0.4% NaCl | Serum |
| February 17, 5 p.m. | 20.5 | 29.9 | 19.4 | 27.2 | 27.9 |
| February 18, 11 a.m. | 21.0 | 29.6 | 20.4 | 26.5 | 28.5 |
| February 18, 5 p.m. | 21.2 | 29.4 | 20.7 | 26.4 | 28.6 |
| | Serum | 0.3% NaCl | Serum | 0.3% NaCl | Serum |
| February 17, 5 p.m. | 33.9 | 30.0 | 53.2 | 54.8 | 20.1 |
| February 18, 11 a.m. | 34.5 | 29.0 | 54.4 | 54.0 | 20.8 |
| February 18, 5 p.m. | 34.8 | 28.7 | 54.8 | 53.5 | 21.0 |

Enough serum for 2 samples for freezing point determinations. First sample read 9.4, 9.2 on potentiometer scale, second read 9.3, 9.3. Standard salt solution, 0.846 per cent NaCl, with $\Delta$ of 0.5°C., gave reading of 14.0 on scale at its freezing point. $\frac{9.3}{14} \times 0.5 = 0.332 = \Delta$ of serum. $\frac{9.2}{14} \times 0.846 = 0.56$ per cent NaCl osmotically equivalent to serum. This agrees with vapor tension method, which shows reversal point between 0.6 and 0.5 per cent NaCl.

low scale arrangement of the Leeds and Northrup type K potentiometer each unit division on the scale represents 0.01 mv. To calibrate the thermocouple a solution of 0.846 per cent NaCl with a calculated $\Delta$ of 0.5°C. was frozen in the small chamber. The average of several determi-

nations showed the null point of the galvanometer when the potentiometer scale read 14.0 divisions. Repeated determinations agree to ± 0.1 scale divisions or ± 0.004°C.; the average of a series of readings is probably within 0.002°C. of the true freezing point. It was also determined that there was no potential difference between the junctions when distilled water was frozen in both chambers. Immediately before the freezing point determinations on the serum in each experiment the potentiometer reading given by the freezing standard NaCl solution was determined in order to be sure that there were no leaks in the thermocouple circuit. The average of the readings was always 14.0 scale divisions. The formula $\frac{R}{14.0} \times 0.5 = \Delta$ gives the $\Delta$ of the serum where R is the average of the potentiometer scale readings with the freezing serum.

One more possible source of error must be considered. After finding in the first experiments that the glomerular fluid column increased in length the possibility arose that osmotically active substances might have entered the glomerular fluid during its stay in the pipette. Although every precaution had been taken to ensure clean rubber tubing, pipette and mercury this possibility had to be investigated. For this purpose a small test tube was filled with 0.7 per cent NaCl solution and covered with a stretched thin rubber dam. The test tube was then fixed horizontally and the tip of the pipette inserted into the salt solution through the rubber dam and from 0.5 to 1 cu. mm. solution drawn into the pipette, which was then withdrawn and washed just as in an animal experiment. The solution was allowed to stay in the pipette for 10 to 15 minutes, at the end of which it was expelled into an 800 $\mu$ capillary receiving tube with a column of salt solution on each side of it dipped from the same test tube. Six such experiments were done and in no case did any of the columns change in length; it was thus shown that the molecular concentration of the glomerular fluid could not have been changed by its stay in the pipette. It is perhaps unnecessary to state that a freshly drawn pipette was used for each control and each animal experiment.

Discussion. It is felt that the results justify the conclusion that the molecular concentration of the glomerular fluid is higher than that of the serum in Necturus. This finding leads one to the same conclusion as to glomerular function as did that by Wearn and Richards of the higher chloride content of the frog's glomerular fluid. The only conclusion possible seems to be that the glomerular membrane is taking an active part in the passage of water and solutes and is putting out a more concentrated solution than is brought to it in the plasma. It is perhaps unnecessary to point out that the molecular concentration of the plasma colloids is a negligible fraction of the total; what slight effect it does exert would of course operate to give the serum a greater concentration than the glomer-

ular fluid. If simultaneous determinations of the glomerular capillary pressure, intracapsular pressure, plasma colloidal osmotic pressure and rate of urine flow show that the pressure relationships are such that a simple hydrostatic filtration can occur, we must accept the view that the glomeruli act as filters but that they are at the same time actively secreting a more concentrated solution. There seems to be nothing incongruous about the idea of a simultaneous operation of these factors.

## SUMMARY

1. The pressure in the lumen of the capsules of functioning glomeruli in Necturus was found to range from 1 to 57 mm. of water.

2. It was established that Barger's vapor tension method for determining molecular concentration is applicable to blood serum.

3. The molecular concentration of the glomerular fluid was shown to be higher than that of the serum in each of twelve experiments.

4. It is concluded that the glomerular membrane actively secretes a solution more concentrated than the plasma. This process is not incompatible with a simultaneous filtration; it is probable that the latter process is operating, but this has not yet been completely proved.

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# THE CARBON DIOXIDE DISSOCIATION CURVE OF NERVE AND MUSCLE

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In attempting to measure the $CO_2$ output of nerve volumetrically in a respirometer (Fenn, 1927), it became obvious that an error was introduced by the rising $CO_2$ tension in the respiration chamber thus causing a progressive increase in the $CO_2$ content of the tissues and a consequent retention of some of the $CO_2$ produced. This error may be considerable at low $CO_2$ tensions and has not infrequently been neglected. To correct for this error the respirometer was modified in such a way that the amount of $CO_2$ taken up by the tissues at different tensions could be measured. In this way a $CO_2$ dissociation curve of the tissues can be drawn in the same manner as for blood. The measurements promised to be of interest also from the more general point of view of the acid base balance of the organism and the part played therein by the tissues, a problem which has recently engaged the interests of several workers (cf. Shaw, 1926; Brockelhurst and Henderson, 1927; Katz and Banus, 1927). Moreover from a knowledge of the combined $CO_2$ and the $CO_2$ tension, the pH of the tissues can be determined. Such information shows to what extent the pH of muscle can change with change of $CO_2$ tension and is important in connection with recent inferences concerning the pH of muscles from measurements made in the expressed juice (Furusawa and Kerridge, 1927; Meyerhof and Lohmann, 1926).

THE METHOD. The apparatus used is shown in figure 1. It consists essentially of a differential volumeter with the necessary apparatus for introducing known mixtures of $CO_2$ and $O_2$ into the respiration chamber. When $CO_2$ is admitted to a muscle previously equilibrated with pure oxygen it is absorbed at a definite rate and the index drop is drawn toward the muscle chamber. Measurements of the rate and extent of movement of the index drop show the rate of absorption and the total amount absorbed. *N* is the respiration chamber which connects through cocks *F* and *G* and the capillary tube with *M*, the control bottle. The nerve or muscle is suspended in the respiration chamber and movements of the index drop are observed until its rate becomes constant. At equilibrium

the drop always moves toward the respiration chamber at a slow rate (1 to 2 mm. in 20 minutes) indicating that the oxygen consumed is slightly greater than the $CO_2$ liberated. When this basal rate is attained, the muscle being in oxygen, a mixture of $CO_2$ in oxygen is introduced. This is done by bringing air pressure to bear upon the mercury surface in bottle *K*. Mercury is thereby forced over into bottle *L* thus expelling the $CO_2$ mixture contained therein into the respiration chamber, escaping through cock *G* which is turned so as to cut off the capillary but to open the bottle

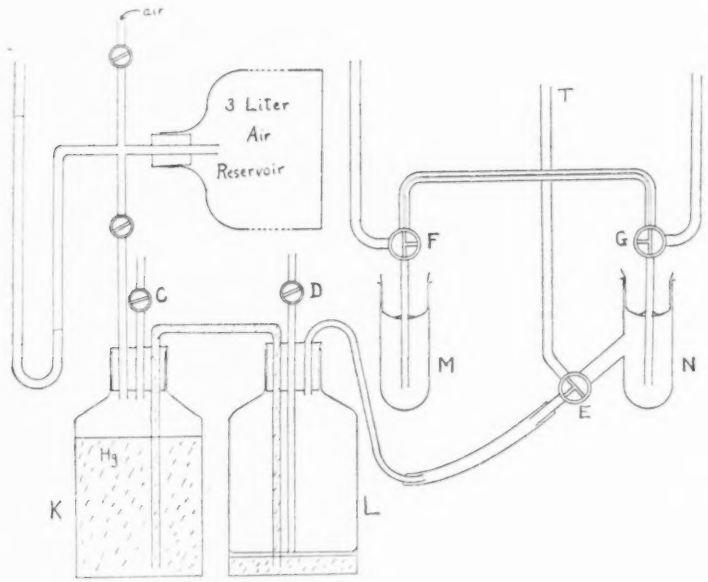


Fig. 1. Arrangement of bottles under the water bath so that the gas inside one of the bottles of a respirometer may be changed without disturbing the temperature and vapor pressure. By thus introducing carbon dioxide the amount absorbed by tissues can be measured.

to the air. Tube *T* is for convenience in flushing out bottle *L* with a new gas mixture. It is found that the $CO_2$ mixture need pass through the respiration chamber for only 5 seconds to make a nearly complete change of gas content. To control the completeness of this change it is only necessary to introduce more of the same mixture after the rate of movement of the index drop shows that absorption is complete. If there is no further absorption when more of the same mixture is introduced it becomes certain that complete equilibrium with the new $CO_2$ mixture has been effected.

By having all the new $CO_2$ mixture completely under water in bottle $L$, it is possible to introduce it into the respiration chamber without changing the temperature or vapor pressure in that bottle, as shown by suitable control experiments. This is not possible if the $CO_2$ mixture is stored in bottles outside of the water bath and is then bubbled through some sort of a coil and saturator under water before entering the respiration chamber. It seems as if in the latter case some of the outside air always reached the respiration chamber before being completely warmed and saturated.

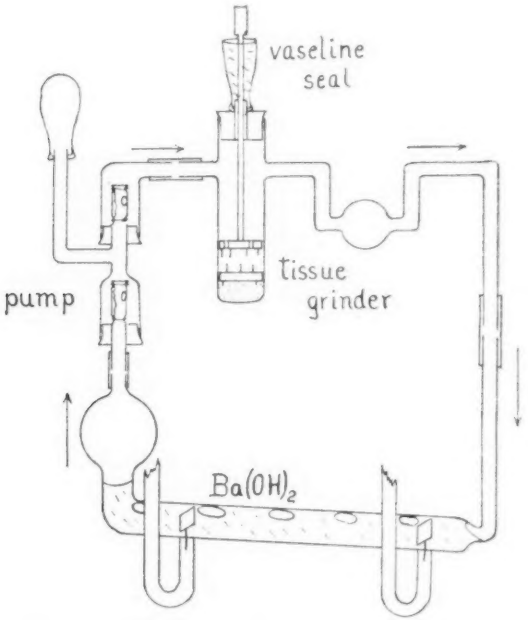


Fig. 2. Apparatus for determining the carbon dioxide content of tissues. The tissue is torn up in the tissue grinder under acid and the carbon dioxide evolved is carried over into the $Ba(OH)_2$ where it is determined by conductivity.

This procedure gives information only concerning the amount of $CO_2$ which a piece of tissue in equilibrium with oxygen will take up when exposed to a given $CO_2$ tension. In order to find the total amount of $CO_2$ in the nerve or muscle it is necessary to analyze the tissue for $CO_2$ when in equilibrium with oxygen. This is easily done, as in Warburg's method, by tipping acid on to the tissue from a side arm of the apparatus after equilibrium has been attained. Instead of using a side arm to contain the

acid before dumping I have used a central well on the bottom of the respirometer bottle. With this arrangement and continuous shaking of the apparatus in the water bath, a complete equilibrium can be attained before dumping the acid, without which very irregular results are obtained. This method has also been used for the direct determination of the amount of combined carbon dioxide in nerves and muscles equilibrated in the respirometer with various tensions of carbon dioxide in oxygen.

Still another method for determining the $CO_2$ content of tissues in equilibrium with different $CO_2$ tensions is illustrated in figure 2. In this

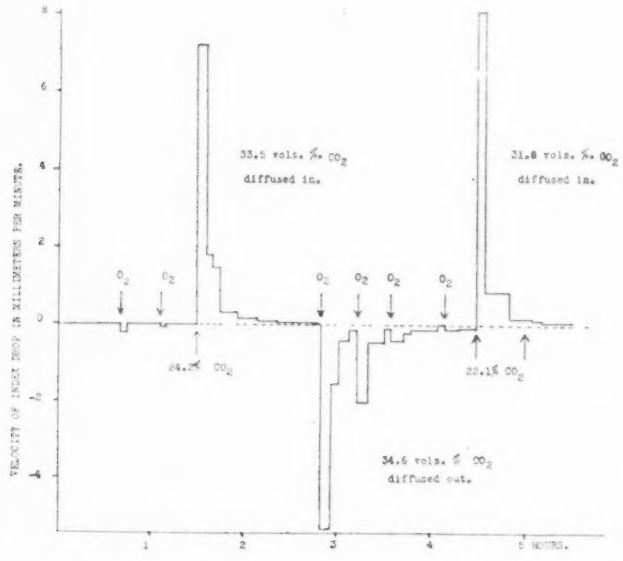


Fig. 3. A typical experiment with the apparatus of figure 1 showing the absorption and evolution of carbon dioxide as the atmosphere surrounding the nerve is changed.

apparatus, air is pumped through the tissue chamber, then through a solution of $Ba(OH)_2$ where the $CO_2$ is removed, and then back to the tissue chamber again. After the $CO_2$ has been removed from the apparatus by pumping the air around before the tissue is introduced, the stopper of the tissue chamber is opened and the tissue in equilibrium with the desired gas mixture is quickly introduced. This can be done without exposing the tissue to the air for more than a few seconds. The amount of $CO_2$ introduced from the air with the tissue is almost negligible except with very small pieces of tissue and can be allowed for if desired. In the tissue chamber is 1 cc. of $CO_2$ free M/10 sulphuric acid. If small pieces of

tissue like nerves are being analyzed they are ground up in sand by the plunger in the tissue chamber. For more bulky tissues like muscle both the bottom of the chamber and the bottom of the plunger are fitted with pins between which the tissue is quickly torn into shreds. The plunger also serves to stir up the sulphuric acid and the air in the chamber. The amount of $CO_2$ liberated is measured by the change in electrical conductivity of the barium hydroxide in a way described elsewhere (Fenn, 1928).

This method possesses the advantage that it can be used for the analysis of tissues taken directly from the body only a few seconds after death. In this way the $CO_2$ content of tissues *in vivo* can be followed.

RESULTS. Most of the results described in this paper were obtained with the first method described above, involving the use of the respirometer. The course of a typical experiment of this sort is shown in figure 3 in which the movements of the index drop are plotted as ordinates against time as abscissae. Arrows indicate the moments when the gas mixture in the chamber was changed. The experiment was performed on two sciatic nerves from a large frog, weighing in all 87 mgm. The nerves were in oxygen at the start and consequently the index drop very nearly stood still. The slight movement toward the nerve chamber indicating a diminution of volume does not show in the diagram. As a control, fresh oxygen was introduced twice in succession at the start without producing any considerable movement of the drop. Then a mixture of 24.2 per cent $CO_2$ in oxygen was introduced and there was a rapid diminution of volume lasting for about an hour and representing the uptake of 33.5 cc. of $CO_2$ per 100 grams of nerve. When this mixture was changed for pure oxygen the index drop moved away from the nerve chamber and indicated a loss of $CO_2$ from the tissues amounting to 34.6 volumes per cent. In this case the demonstration was made more complete by introducing oxygen 4 times but ordinarily two such introductions suffice to remove practically all of the $CO_2$. Another $CO_2$ mixture was then introduced which on analysis proved to contain 22.1 per cent $CO_2$. The same mixture of $CO_2$ when reintroduced a second time, as indicated by the arrow, had no effect upon the rate of movement of the drop showing that in the 5 seconds allowed the change in gas mixture had been practically complete. If the exchange is not quite complete it of course makes more difference when $CO_2$ is being removed than when it is being introduced. This explains the relatively large movement obtained when oxygen is introduced a second time.

*The $CO_2$ dissociation curve of nerve.* The $CO_2$ dissociation curve of nerve, as obtained by the above method is plotted in figure 4 (upper graph), ordinates being the volumes per cent of $CO_2$ combined, while abscissae are tensions of $CO_2$ in mm. Hg. The curve represents the tissue

as containing a certain amount of $CO_2$ at a $CO_2$ tension of zero. Strictly speaking this condition was not attained, for the tension is never quite 0 even in oxygen. This value was obtained by dumping acid on the nerve when in equilibrium with oxygen, and was confirmed by the conductivity method. The nerves used for these determinations were dissected out under exactly the same conditions as those used for measurements of the oxygen consumption during stimulation and may, therefore, be considered irritable and in good condition. The points with a dot were obtained by measuring the contraction of volume when the $CO_2$ was being absorbed; those marked with a cross were obtained when oxygen was introduced

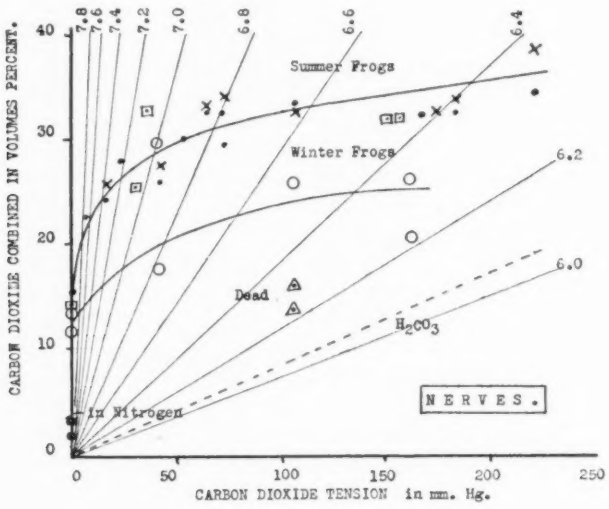


Fig. 4. The carbon dioxide dissociation curve of frog sciatic nerves

and the $CO_2$ diffused out again. The agreement between these two determinations seems very satisfactory. The dotted line represents the amount of $CO_2$ which would dissolve in the nerve if no extra base were made available by the dissociation of proteins or other weak acids. It is plotted on the assumption that the nerve contains 80 per cent water (as found by analysis) and that the solution of $CO_2$ in the remaining solids would be negligible. The amount which is assumed on this basis to be physically dissolved in the nerve has been deducted from the values experimentally observed in order to obtain the amount of bound $CO_2$, values of which are plotted. The curve shows a slight upward slope even at high $CO_2$ tensions. If the amount of $CO_2$ physically dissolved in the

nerve were somewhat greater than the value assumed, then this slope would be correspondingly less.[1]

In order to confirm this slope I have studied the solubility of carbon dioxide in nerves soaked in M/10 HCl to exhaust all their buffers. Four such nerves were placed in a cup in the bottom of a respirometer bottle and equilibrated with 20 per cent $CO_2$ in oxygen. Mercury was then introduced through a stopcock in the stopper, completely filling the cup and submerging the nerves. After thoroughly flushing out the carbon dioxide with a current of oxygen the apparatus was equilibrated in the water bath until there was no further volume change as indicated by the kerosene drop. The respirometer was then tipped so that the mercury ran out of the cup and exposed the nerves to oxygen. The prompt evolution of $CO_2$ was followed by means of the kerosene drop. The results of two such analyses showed that the nerves absorbed as much $CO_2$ as 78 and 82 per cent respectively of their weight of water. This obviously agrees well with my assumed value of 80 per cent. Since the nerves were somewhat swollen (25 per cent) from the treatment with HCl, the true figure for normal nerve would be if anything less.

The upper graph of figure 4 was obtained by measurements on summer frogs. A very similar curve was obtained (unpublished) with spring frogs. The points marked with squares were obtained by dumping acid on nerves equilibrated in the respirometer at different tensions of $CO_2$. No correction for dissolved $CO_2$ was therefore necessary. The nerves in this case were from frogs received in the laboratory at the end of March and kept in water at about 4°C. Other frogs received in November and kept similarly in the cold room gave somewhat lower values as indicated by the lower graph. This graph is labelled "winter frogs" although it is not certain that the low values represent a true seasonal variation. In the latter case analyses were made by the conductivity method (fig. 2).

The diagonals of figure 4 represent pH values as calculated from the Henderson-Hasselbalch equation using a value of 6.17 for $pK_1$ as given for human blood plasma at 22°C. by Cullen, Keeler and Robinson (1925). If the $CO_2$ tension inside a living frog at rest at 20°C is 14 mm. Hg (see p. 216) then the pH inside the nerve should be 7.4 according to the diagram.[2] Meyerhof and Gerard (cf. Gerard, 1927) were unable to detect

[1] In Warburg's method for simultaneous determination of $CO_2$ and $O_2$ manometrically which takes advantage of the wide difference between the solubilities of $CO_2$ and of $O_2$ this curve should strictly be considered. At 5 per cent $CO_2$ the solubility of $CO_2$ (including that combined) in nerve and muscle tissues is greater than its solubility in water. Neglect of this might introduce a small error depending upon the amount of tissue used.

[2] In determining the pH from the $H_2CO_3$ to $NaHCO_3$ ratio it is fully realized that there may be inequalities in the distribution of these substances between cells and lymph which would make the pH vary. The pH value stated is merely an aggregate

any increase in lactic acid in nerve due to stimulation and likewise I have been unable to find any consistent change in the $CO_2$ combining capacity during stimulation. On the other hand some dead nerves which had been kept at room temperature for 24 to 48 hours after dissection showed distinctly low values as indicated by the figure (triangles) and two nerves kept for 2 to 4 hours in nitrogen showed 1.9 and 3.3 volumes per cent $CO_2$ respectively when acid was dumped on them in the respirometer, instead of the normal value of 14 volumes per cent found in oxygen. This is

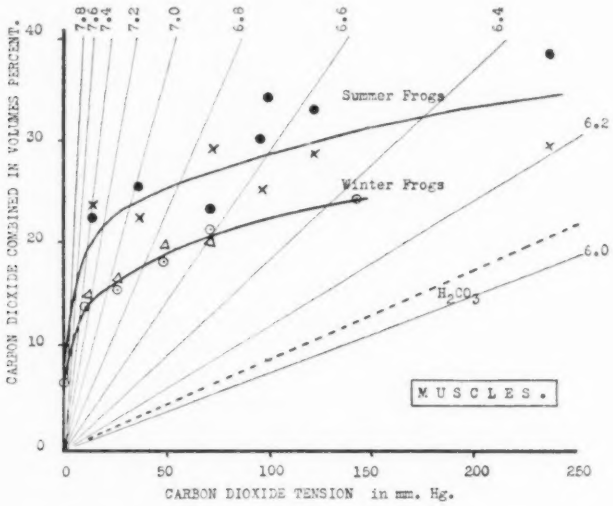


Fig. 5. The carbon dioxide dissociation curve of frog muscles

obviously explained by a formation of lactic acid as Meyerhof and Gerard have found by analysis (Gerard, 1927).

*The $CO_2$ dissociation curve of muscles.* Similar curves for muscles are plotted in figure 5. Both graphs in this case were obtained by the volumetric method (fig. 1). Circles indicate points obtained when $CO_2$ diffused in, crosses and triangles when $CO_2$ diffused out. Again it has been assumed that $CO_2$ dissolves in the muscle as $H_2CO_3$ as it would in 80 per cent of its volume of water, this being the figure for the water content of frog skeletal muscle given in *Tabulae Biologicae* (vol. iii, p. 447). The

value but seems nevertheless to be quite as good as values obtained from measurements made directly in minced muscles or muscle extracts where the muscle structure is completely destroyed. The value of $pK_1$ for tissues may also be different from that assumed.

absorption coefficient is 0.829 at 22°C. (Landolt Börnstein). The lower graph was obtained from measurements on large spring and summer frogs which were kept in a cold room at about 4°C. without any opportunity for feeding. Later measurements on a batch of smaller frogs kept at room temperature gave higher values. These two graphs are distinguished as summer and winter frogs for convenience although it is not quite certain

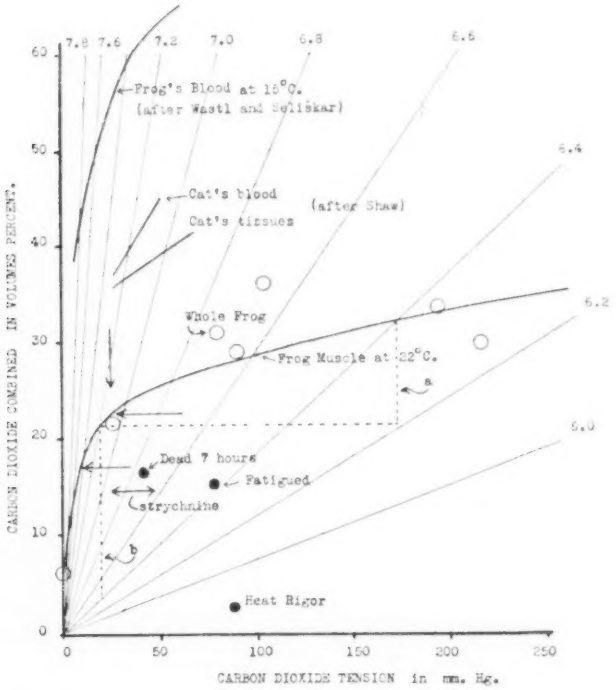


Fig. 6. The carbon dioxide dissociation curve of frog blood and muscles, together with other determinations of combined carbon dioxide under various conditions for comparison.

that this was the only difference between them. The fact that both nerves and muscles seem to show lower values for winter animals is in agreement with the findings of Meyerhof and Lohmann (1926) that muscles of winter frogs show a lower buffering capacity than muscles of summer frogs.

The $CO_2$ dissociation curve for muscles of summer frogs is reproduced in figure 6 for comparison with other values. The points marked there

with circles were obtained by the conductivity method from autumn frogs and are in substantial agreement with the previous curve. One of these points was obtained by equilibrating the whole live frog with the desired mixture of $CO_2$ and oxygen. After about three quarters of an hour in this atmosphere the frog was rapidly killed, one leg was clamped tightly with a hemostat to cut off the circulation, and the gastrocnemius muscle was removed with all possible speed and plunged into the tissue chamber of the conductivity apparatus, shredded in acid, and the conductivity change read. This value so obtained is in agreement with other results. Two frogs while resting quietly at room temperature in ordinary air were similarly killed and their muscles analyzed. The results are marked by the horizontal arrows, the values being 17 and 22.5 volumes per cent. Assuming the validity of the dissociation curve as plotted, and correcting my figures for $CO_2$ absorbed as $H_2CO_3$, they would indicate a resting $CO_2$ tension inside the frog of about 15 to 20 mm. Hg. This value is only slightly lower than the point marked by the vertical arrow, 25.5

TABLE 1

*pH of skeletal muscle*

| OBSERVER | RESTING | FATIGUED | RIGOR | |
|---|---|---|---|---|
| Pechstein (1914) | 7.23 | 6.84 | 6.3 –6.4 | Frog |
| Schade, Neukirch and Halpert (1921) | 7.3 | 6.7 | | |
| Meyerhof and Lohmann (1926) | 7.11–7.30 | 6.19–6.62 | 5.87–6.39 | Frog 15°C. |
| Furusawa and Kerridge (1927) | 7.04 | 6.26 | 6.02 | Cat 0°C. |
| Fenn | 7.2–7.3 | 6.6–6.8 | 6.0 | Frog 20°C. |

mm. Hg, which is the estimate made by Wastl and Seliškar (1925) from studies on the blood of R. catesbiana. This indicates a resting pH of the muscles of 7.2 to 7.3. From measurements made on muscles fatigued by prolonged stimulation a pH of 6.4 was found at 77 mm. Hg $CO_2$ tension (see fig. 6). At normal $CO_2$ tensions this would correspond to a pH of 6.6 to 6.8. This figure is in agreement with a similarly low $CO_2$ content found in a muscle from a frog in strychnine convulsions (indicated in fig. 6 by a double arrow because the $CO_2$ tension was unknown). An observation on a muscle in heat rigor after equilibration at 88 mm. Hg of $CO_2$, (see fig. 6) shows such a low $CO_2$ combining capacity that the pH must have been below 6.0. These values compare well with other values obtained by direct measurement of the pH in minced muscles and muscle extracts as found in the literature (table 1). My own values in table 1 are based on comparatively few analyses.

Wastl and Seliškar 1925 have published a $CO_2$ dissociation curve for the blood of Rana catesbiana which is plotted, together with my curve for frog muscle (R. pipiens) in figure 6. The $CO_2$ combining capacity of

muscle is evidently very much less than that for blood when equal weights of blood and muscle are compared. Shaw (1926) has measured the total amount of $CO_2$ stored by a cat for a given rise in $CO_2$ tension in the lung alveoli. Knowing the amount of $CO_2$ which could be combined in the blood of the animal over this range of $CO_2$ tension, he was able to estimate the amount taken up per gram of tissue. In a typical experiment he found that 87.4 per cent of the total $CO_2$ absorbed was held in the tissues, but when equal volumes of tissue and blood were compared it was found that the blood was over twice as effective as a buffer. The slopes of the $CO_2$ dissociation curves of blood and tissues so found were respectively 0.364 and 0.147 volume per cent absorbed per mm. Hg rise in $CO_2$ tension. Lines having such slopes have been drawn in figure 5 for comparison with

TABLE 2

*Buffer values of blood and tissues*

| (1) TISSUE | (2) pH | (3) $BHCO_3$ | (4) OTHER BUFFERS | (5) $CO_2$ ESCAPE | (6) TOTAL | (7) REFERENCE |
|---|---|---|---|---|---|---|
| Frog muscle, 22°C | 7.2–6.4 | 0.0040 | 0.0061 | 0.0100 | 0.020 | Fig. 6 |
| Frog nerve, 22°C | 7.2–6.4 | 0.0047 | 0.0052 | 0.0122 | 0.022 | Fig. 5 |
| Human blood, 37°C | 7.8–7.0 | 0.0022 | 0.0206 | 0.0167 | 0.039 | Van Slyke, 1922 |
| Frog blood, 15°C | 7.8–7.0 | 0.0029 | 0.0172 | 0.0240 | 0.044 | Wastl and Seliškar, 1925 |
| Frog muscle, 15°C? | 7.3–6.46 | | | | 0.048 | Meyerhof and Lohmann, 1926 |
| Frog muscle, 15°C? | 6.46–5.99 | | | | 0.018 | Meyerhof and Lohmann, 1926 |
| Cat skeletal muscle, 0°C. | 7.4 | | | | 0.025 | Furusawa and Kerridge, 1927 |
| Cat skeletal muscle, 0°C. | 6.5 | | | | 0.052 | Furusawa and Kerridge, 1927 |

my own curves for muscle and that of Wastl and Seliškar (1925) for blood. The absolute position of these lines on the diagram is arbitrary but the slope is significant and each agrees roughly with the slope of curves shown for the frog over the physiological range. Certainly tissues are less effective as buffers than is blood, volume for volume.

My results are not in agreement with the very low values for the slope of the $CO_2$ dissociation curve for the body as a whole reported by Brocklehurst and Henderson (1927). Possibly they allowed insufficient time for equilibration.

Van Slyke (1922) has formulated a method for measuring buffer values and has given a calculation of the buffer value of blood from its $CO_2$ dissociation curve. It has seemed worth while to make a similar calculation for muscle and nerve for purposes of comparison. The results are

collected in table 2. I have chosen to calculate the buffer value for muscle and nerve between pH values of 7.2 and 6.4 this being approximately the change caused by fatigue. Column 3 of table 2 gives the buffer value due to $BHCO_3$ alone calculated according to the formula 12 of Van Slyke as he has done it for blood on p. 562 of his article. Column 4 gives the buffer value of the other weak acids. This is calculated from the rise of the $CO_2$ dissociation curve between its intersections with the pH 7.2 and the pH 6.4 diagonals, as described by Van Slyke (cf. dotted line *a* in fig. 6). The sum of columns 3 and 4 gives the value of $\beta$ defined by Van Slyke as the "number of gram equivalents of strong acid or alkali taken up per unit change in pH," or $\frac{dB}{d\text{pH}}$. This buffer value does not measure, however, the total ability of a muscle to neutralize lactic acid, for bicarbonate can be broken up and $CO_2$ can escape. The amount of acid which can be disposed of in this way between the pH values 7.2 and 6.4 without change in $CO_2$ tension can be estimated likewise from the $CO_2$ dissociation curve as indicated by the dotted line *b* in figure 6. This distance in figure 6 represents 21–3, or 18 volumes per cent. This corresponds to a loss of 18/2240 or 0.008 mol. per liter of muscle for a change of 0.8 pH. This indicates a change of 0.01 mol. per unit change in pH (column 5). For muscle the total true buffer value, $\beta$, is thus 0.004 plus 0.006 (columns 3 and 4) or 0.01. Hence it appears that over this range of pH and for this muscle *one-half of the lactic acid produced in fatigue is neutralized by the liberation of $CO_2$ from bicarbonate and the remainder by the buffer value of the bicarbonate itself and of the other weak acids present in muscle.*[3]

Meyerhof (1922) has estimated that the preformed $CO_2$ in muscles was sufficient to neutralize only $\frac{1}{10}$ of the lactic acid formed but this estimate was based on analyses for $CO_2$ in muscles equilibrated with pure oxygen (presumably) which would of course be much too low. Meyerhof states also that sartorius muscles (at zero $CO_2$ tension presumably) contain 8.5 to 13.8 vols. per cent of $CO_2$ most of which disappears in fatigue. This is in good agreement with my results.

Other values in table 2 have been similarly calculated. The values for human blood were taken from Van Slyke, with the exception of the estimate of the $CO_2$ escape which has been calculated from his $CO_2$ dissociation curve for human blood (Van Slyke, 1921) in the manner described. The figures from Meyerhof and Lohmann, 1926, were based upon measurements of lactic acid content and pH of muscle extracts of R. esculenta, autumn frogs. The values given in table III of these authors were averaged and the buffer value was calculated from corresponding values for change in pH and change in base or lactic acid concentration. The values

[3] This statement would require modification if appreciable amounts of base are formed during contraction as from ammonia or phosphocreatin.

taken from Furusawa and Kerridge (1927) for the buffer value of cat's gastrocnemius were taken directly from figure 3 of their paper after dividing by 100 to account for a change of units. In this case acid and alkali were added directly to minced muscle at 0°C. and the pH was measured by the glass electrode. It is difficult to compare my results directly with the somewhat higher values of Furusawa and Kerridge and of Meyerhof and

TABLE 3

*Rate of diffusion of carbon dioxide into nerve and muscle*

Percentages not absorbed at various times after exposure. Comparison of calculated and observed values.

| EXPT. 1. NERVE | | | EXPT. 2. NERVE | | | EXPT. 3. NERVE | | |
|---|---|---|---|---|---|---|---|---|
| Time | Per cent calculated | Per cent observed | Time | Per cent calculated | Per cent observed | Time | Per cent calculated | Per cent observed |
| *minutes* | | | *minutes* | | | *minutes* | | |
| 0 | 100 | 100 | 0 | 100 | 100 | 0 | 100 | 100 |
| 1 | 68 | 64 | 1 | 67 | 69 | 1 | 72 | 81 |
| 2 | 56 | 55 | 2 | 56 | 58 | 2 | 65 | 68 |
| 3 | 49 | 46 | 3 | 48 | 48 | 3 | 60 | 61 |
| 4 | 42 | 40 | 4 | 41 | 40 | 4 | 53 | 56 |
| 6 | 31 | 31 | 6 | 31 | 30 | 6 | 47 | 46 |
| 9 | 21 | 22 | 9 | 21 | 20 | 8 | 40 | 39 |
| 24 | 3 | 7 | 19 | 6 | 6 | 12 | 30 | 30 |
| 30 | 1 | 6 | 65 | 1 | 1 | 31 | 8 | 12 |
| | | | | | | 37 | 6 | 9 |
| EXPT. 4. NERVE | | | EXPT. 5. NERVE | | | EXPT. 6. MUSCLE | | |
| 0 | 100 | 100 | 0 | 100 | 100 | 0 | 100 | 100 |
| 1 | 63 | 64 | 1 | 62 | 66 | ½ | 76 | 81 |
| 2 | 50 | 49 | 2 | 49 | 49 | 1 | 71 | 73 |
| 3 | 41 | 39 | 3 | 40 | 39 | 2 | 63 | 63 |
| 4 | 34 | 32 | 4 | 32 | 32 | 3 | 56 | 54 |
| 5 | 28 | 28 | 6 | 22 | 22 | 5 | 46 | 45 |
| 6 | 24 | 24 | 9 | 13 | 14 | 7 | 39 | 37 |
| 10 | 12 | 15 | 13 | 6 | 10 | 9 | 33 | 33 |
| 14 | 6 | 11 | | | | 11 | 27 | 30 |
| 25 | 4 | 7 | | | | 18 | 17 | 21 |
| 28 | 1 | 4 | | | | 32 | 5 | 7 |

Lohmann because no mention is made by these authors of the $CO_2$ tension in the extracts used. For purposes of comparison it is assumed that the $CO_2$ tension was constant in their experiments. In this connection it is difficult to understand the statement of Pechstein (1914), who measured the pH of extracts of boiled muscle, that $CO_2$ loss was not an appreciable factor in changing the pH.

*The rate of absorption of carbon dioxide.* For a determination of the theoretical rate of diffusion of a gas into a nerve, the nerve may be treated without serious error as a cylinder of indefinite length and average diameter. It should therefore be possible to show that the rate of entrance or carbon dioxide agrees with the theoretical rate and it should be possible to make some estimate of the diffusion constant of $CO_2$ in nerve from these rates.

The rate of diffusion of any substance into a cylindrical body of indefinite length and radius $a$, is given by the following formula taken from the calculations of Andrews and Johnston (1924).

$$\frac{Q}{Q_1} = 1 - 4\left\{\frac{1}{5.783}\epsilon^{-\frac{kt}{a^2}\times 5.783} + \frac{1}{30.47}\epsilon^{-\frac{kt}{a^2}\times 30.47} + \text{etc.}\right\} \qquad (1)$$

$Q_1$ is the total amount of the substance which must diffuse before equilibrium is reached. $Q$ is the amount which has diffused in time $t$. $k$ is the diffusion constant of the substance concerned. Where only one term of the series needs to be considered this equation may be written

$$\frac{kt}{a^2} = -0.06375 - 0.3983 \log\left(1 - \frac{Q}{Q_1}\right) \qquad (2)$$

To apply this equation to the diffusion of $CO_2$ into nerve, movements of the index drop have been observed at different times after introducing $CO_2$ into the nerve chamber, and the per cent $1 - \frac{Q}{Q_1}$ which has not diffused in at various times, $t$, is determined. Thence values of $\frac{k}{a^2}$ are determined (equation 2). These values are not constant during the first few minutes of diffusion, because the first term has been neglected, nor are they constant during the final stages of the diffusion, the reaction being, for some reason, more prolonged than can be accounted for completely on diffusion alone. $\frac{k}{a^2}$ reaches a fairly constant value, however, for a large part of the diffusion and this figure is chosen for calculation (by equation 1) of the theoretical values of $1 - \frac{Q}{Q_1}$. In table 3, these values, calculated from a number of experiments, are compared with the values actually observed. The close agreement so revealed, between experiment and theory, is sufficiently good, if the last 10 per cent of the diffusion can be excepted, to justify the belief that the diffusion constant, $k$, so calculated might be fairly close to the correct figure. One error in determining $k$ seems to lie in the uncertainty in $a$ the radius of the nerve. Unfortunately the length of the nerves used was not measured in every instance; from an estimated figure for the length (5 cm.) and the weight of nerve, one can calculate a diameter which has

been used in table 4 for calculating the diffusion constant, $k$. The average value of $k$ for nerve is $7.1 \times 10^{-5}$ cm.²/min. Similar calculations for muscles yield somewhat higher and less regular results, the average being $11.7 \times 10^{-5}$ cm.²/min. In the case of the muscles, due perhaps to their odd shapes, the formula does not fit the experimental curves as accurately as in the case of nerves. The divergence occurs in the first few minutes when the per cent of $CO_2$ still unabsorbed is about 10 per cent greater than the theoretical figure.

In table 5, I have collected together the diffusion coefficients of carbon dioxide and oxygen in various media. In rubber and water the relative diffusion coefficients are nearly as 1 to 1.18 or inversely as the square roots

TABLE 4

*Diffusion coefficients of carbon dioxide in nerve and muscle*

| EXPT. | $CO_2$ | $\frac{K}{\alpha_2}$ | VOL. NERVE | $a^2$ | $K$ |
|---|---|---|---|---|---|
| | | | Nerve | | |
| | *per cent* | | *cm.³* | *cm.²* | *cm.²/min.* |
| 1 | 8.5 | 0.023 | 0.048 | 0.0031 | $7.1 \times 10^{-5}$ |
| 2 | 9.3 | 0.023 | 0.046 | 0.0029 | 6.7 |
| 3 | 3.1 | 0.0118 | 0.049 | 0.0031 | 3.7 |
| 4 | 24.2 | 0.031 | 0.042 | 0.0027 | 8.4 |
| 5 | 29.2 | 0.033 | 0.046 | 0.0029 | 9.6 |
| | | | Average = $7.1 \times 10^{-5}$ cm.²/min. | | |
| | | | Muscle | | |
| 6 | 12.5 | 0.0145 | 0.032 | 0.005 | 7.2 |
| 7 | 3.5 | 0.01 | 0.141 | 0.0112 | 11.2 |
| 8 | 13.0 | 0.009 | 0.072 | 0.009 | 8.1 |
| 9 | 9.4 | 0.014 | 0.144 | 0.0115 | 16.1 |
| 10 | 16.0 | 0.02 | 0.068 | 0.008 | 16.0 |
| | | | Average = $11.7 \times 10^{-5}$ cm.²/min. | | |

of the densities of the two gases. According to my figures the diffusivity of carbon dioxide in muscle and nerve is higher than in rubber but considerably lower than in water. The comparison between my figures and those of Krogh (1919) is not so straightforward, for Krogh did not really measure directly the diffusivity but rather the permeability; or the amount of gas diffusing through a sq. cm. of membrane under a constant diffusion gradient of 1 atmosphere per micron. The units of Krogh's "diffusion constants" were therefore $\frac{\text{cm.}^2}{\text{min. atmosphere}}$. He expressed his results this way because he did not know the solubilities of the gases in the tissues. If his values are divided by the solubilities, $\frac{\text{cm.}^3}{\text{cm.}^3\text{atmosphere}}$ the units be-

come cm.²/min. as in table 5. To calculate the diffusivity from Krogh's figures I have therefore had to assume a value for the solubility. For oxygen I have used 0.03145, which is the solubility of oxygen in water at 20°C. and 760 min. pressure. For the solubility of carbon dioxide one is faced with the difficulty that some of the $CO_2$ absorbed is combined and diffuses as $HCO_3'$. As such it would not diffuse across cell membranes as readily as $CO_2$ but would nevertheless contribute something to the diffusion as a whole. To the extent that it does contribute to the diffusion, the combined $CO_2$ should be included in the "solubility." Since the combined $CO_2$ varies with the tension, and since the tension fell from 1 atmosphere (presumably) on one side of Krogh's membrane to very low values on the other, one must take some sort of an average. If the combined $CO_2$ is included, the "solubility" varies from about 1 (cc. per gram tissue per

TABLE 5

*Diffusion coefficients in cm.²/min. of oxygen and carbon dioxide in various media*

| MEDIUM | TEMPERATURE | OXYGEN | CARBON DIOXIDE | |
|---|---|---|---|---|
| Water | 16°C. | $113 \times 10^{-5}$ | $95 \times 10^{-5}$ | Hüfner, 1897 |
| Rubber | 17°C. | 5.7 | 5.1 | Daynes, 1920 |
| Connective tissue | 20°C. | 37 | 20–27 | Krogh, 1919 |
| Muscle | 20°C. | 45 | | Krogh |
| Muscle | 22°C. | | 11.7 | Fenn |
| Nerve | 22°C. | | 7.1 | Fenn |
| Theoretical ratio | | 1.18 | 1.0 | Inversely as $\sqrt{\text{density}}$ |

The figures of Hüfner and of Daynes have been multiplied by $\frac{1}{1440}$ and 60 respectively to change the units to minutes. Krogh's figures have been multiplied by $10^{-4}$ to change microns to centimeters and divided by the absorption coefficient.

atmosphere) at 760 mm. Hg to 2.3 at 100 mm. Hg and 7.4 at 25 mm., etc. As a guess therefore I have taken an average value of 1.5 to 2.0 which gives a diffusivity ($k$) of 20 to 27 $\times 10^{-5}$. Two factors might help to explain my lower values of 7.1–11.7 $\times 10^{-5}$. (1) Part of the surface of the nerves and muscles was in contact with the glass and therefore not available for diffusion as the formula assumes. (2) The diffusivity of the $HCO_3'$ may be much less than that of $CO_2$. In the case of the nerves high $CO_2$ tensions gave high values for $k$ (table 4), perhaps because at high tensions relatively less of the $CO_2$ absorbed is combined.

Further studies on the diffusion constants of $CO_2$ in buffered and unbuffered media are planned. The values here reported must be regarded as mere approximations.

SUMMARY

1. Methods are described for measuring the amount of $CO_2$ absorbed by nerves and muscles at various $CO_2$ tensions.

2. Variations in the $CO_2$ combining capacity of nerves and muscles have been measured in fatigue, death, rigor and anoxemia.

3. From the data obtained, inferences are drawn concerning the pH inside the tissues in different conditions.

4. In both nerve and muscle the $CO_2$ combining capacity seems to be less in winter frogs than in summer frogs; it is $\frac{1}{3}$ to $\frac{1}{2}$ as great in such tissues as in blood.

5. The "buffering capacity" (of Van Slyke) has also been calculated for comparison with the slightly higher values obtained by others by direct pH readings in minced or extracted muscles.

6. It can be estimated that about half the lactic acid formed in muscle is neutralized by liberation and escape of $CO_2$, the remainder combining with base from weaker acids.

7. The rate at which $CO_2$ diffuses into nerves agrees with that predicted from Fick's law of diffusion, and from the results, an estimate can be made of the diffusion coefficient, which is $7.1 \times 10^{-5}$ cm.$^2$/ min. in nerves and $11.7 \times 10^{-5}$ cm.$^2$/min. in muscle.

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# SIMULTANEOUS STUDY OF THE CONSTITUENTS OF THE SWEAT, URINE AND BLOOD; ALSO GASTRIC ACIDITY AND OTHER MANIFESTATIONS RESULTING FROM SWEATING

## VII. Amino-Acids

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In one of our previous papers of this series we have offered data on the total non-proetin nitrogen in the simultaneous study of the sweat, urine and blood, and in subsequent articles we have discussed some of the individual nitrogenous constituents. The urea and ammonia nitrogen have already been reported, and it is our purpose in this article to deal similarly with the amino-acids. In this connection it might be well to state that we are only concerned with those nitrogenous bodies that are known to be in the sweat and consequently are found in the urine and blood as well.

We are not the first to have discovered amino-acids in the sweat, for it has been reported in a few experiments by Embden and Tachau (1) where they claim to have found from 3.65 to 4.2 mgm. per 100 cc. Furthermore, from their observations they maintain that serin or beta-hydroxy-alpha-amino-propionic is the chief amino acid in the sweat. Neuberg (2) also speaks of amino acids in the sweat. It has been our purpose to extend this work further and perhaps make a more intensive study of the problem by more modern methods.

The method for producing the sweat was that previously reported by one of us (3) while the method of Folin (4) was used for the determination of the amino acid nitrogen. For the making of the protein-free blood filtrate the method of Folin and Wu (5) was adopted, but in the later work a modification of the same by Haden (6) was brought into use which seemed to be a check on the former and has the advantage of simplicity. A few determinations were also made by the well-known Van Slyke (7) apparatus.

This, like much of our work, was done in two periods. The first part represents the work of the summer of 1926 and is reported in table 1, while the second constitutes the work of the summer of 1927 and is reported in

table 2. In both of these tables we are reporting a simultaneous determination of the amino-acid nitrogen of the blood and urine with that of the sweat. As for the blood a control sample was drawn, deproteinized and tested just before sweating and in like manner a second sample immediately after retiring from the sweat cabinet. Also two samples of urine

TABLE 1

| SUBJECT | BLOOD 1 | BLOOD 2 | SWEAT | URINE 1 | URINE 2 |
|---|---|---|---|---|---|
| F. C. | | | 2.9 | | |
| J. M. | 7.0 | 6.5 | 2.88 | 13.7 | |
| R. B. | 6.4 | 6.7 | 2.22 | | |
| M. A. | 6.6 | 6.2 | 3.00 | | |
| F. C. | 7.3 | 6.4 | 2.6 | 13.9 | 18.5 |
| J. A. | 7.2 | 7.6 | 3.7 | 12.2 | 9.3 |
| I. H. | 6.7 | 6.9 | | 17.5 | 14.8 |
| W. K. | 6.1 | 4.8 | 1.8 | 8.5 | 10.8 |
| I. R. | 8.3 | 7.3 | 2.4 | 21.2 | 18.1 |
| J. B. | | | 2.8 | 18.2 | 12.5 |
| F. C. | | | 1.6 | | |
| C. H. | 7.5 | | | 21.4 | 14.9 |
| F. C. | | | 1.9 | 11.8 | 14.6 |
| J. A. | 7.8 | 7.7 | 4.1 | 7.9 | 10.9 |
| J. M. | 6.3 | 5.9 | 2.1 | 12.4 | 17.9 |
| W. K. | 6.8 | 6.6 | 1.9 | 9.0 | 10.1 |
| F. C. | | 7.7 | 4.0 | 15.7 | 16.2 |
| I. R. | 7.6 | 7.8 | 4.7 | 15.0 | 14.3 |
| W. K. | 8.0 | 7.9 | 4.0 | 20.2 | 20.4 |
| C. H. | 7.4 | 7.6 | 4.2 | 20.0 | 20.3 |
| F. C. | | | 2.7 | | |
| J. A. | 7.8 | 7.1 | 2.7 | 9.0 | 7.9 |
| I. R. | 6.3 | 7.1 | 3.8 | 23.3 | 23.0 |
| F. C. | 6.7 | 7.1 | 2.2 | 20.6 | 22.5 |
| O. H. | 6.3 | 7.0 | 1.9 | 11.0 | 12.4 |
| J. A. | 7.0 | 6.6 | 3.1 | 24.3 | 22.3 |
| F. C. | 6.8 | 6.7 | 3.3 | 20.0 | 20.8 |
| W. K. | 6.8 | 6.8 | 1.6 | 10.0 | 10.5 |
| C. H. | 7.4 | | 3.6 | 18.1 | 18.7 |
| O. H. | 6.8 | | 2.4 | 15.1 | 18.1 |

The above results are in terms of milligrams per 100 cc.

were taken and tested in a similar manner with the exception that the second sample of urine was not voided until about 20 minutes after sweating.

It will be noted in our tables that there are a few determinations that are lacking. These omissions were unavoidable due to one cause or another but mainly due to the subject not being able to yield sufficient amount of the sample to satisfy all of the collaborators who were making the various determinations.

TABLE 2

| SUBJECT | BLOOD 1 | BLOOD 2 | SWEAT | URINE 1 | URINE 2 |
|---|---|---|---|---|---|
| O. B. | 8.58 | 8.85 | 4.70 | | |
| F. H. | 6.33 | 7.10 | 2.88 | | |
| F. B. | | | 4.00 | | |
| O. H. | 6.73 | 3.04 | 2.00 | | |
| F. C. | 6.14 | 6.48 | 1.55 | | |
| I. R. | 6.35 | 5.53 | 2.47 | 12.98 | 17.09 |
| J. L. | 5.64 | 7.21 | 3.38 | 13.88 | 21.05 |
| A. M. | | | | 16.39 | |
| F. C. | 7.84 | 7.93 | 2.78 | | |
| O. B. | 6.69 | 7.30 | 3.30 | | |
| O. H. | | | 4.02 | | |
| I. R. | 9.21 | 6.51 | 4.70 | 18.18 | 25.85 |
| F. C. | | | 2.51 | 15.26 | 19.04 |
| O. H. | 6.86 | 7.21 | 4.84 | 21.52 | 23.95 |
| R. O. | 7.86 | 8.48 | 4.50 | | |
| O. B. | 7.56 | 6.15 | 2.83 | 12.57 | 11.69 |
| F. C. | 7.43 | 7.87 | 2.68 | 16.08 | 14.22 |
| F. H. | 7.02 | 7.96 | 2.75 | | |
| A. S. | 6.22 | 5.89 | | 19.70 | 23.37 |
| O. H. | | | 2.77 | | |
| F. B. | 6.83 | 6.93 | 4.39 | | |
| L. P. | 5.14 | 5.55 | 2.30 | 18.69 | 26.66 |
| I. R. | 6.73 | 7.36 | 3.77 | | |
| O. B. | 8.86 | 8.75 | 1.62 | 21.85 | 23.12 |
| J. L. | 7.86 | 8.23 | | | |
| F. H. | 6.82 | 7.77 | 1.93 | 12.50 | 12.12 |
| J. L. | 6.42 | 7.03 | | 15.81 | 15.15 |
| F. B. | 7.69 | 8.09 | 2.56 | 23.80 | 21.27 |
| O. H. | 7.44 | 7.86 | 2.72 | 22.72 | 28.57 |
| F. C. | 4.94 | 5.60 | 5.40 | 20.00 | 23.52 |
| I. R. | 7.00 | 6.48 | 4.96 | 24.53 | 24.09 |
| O. H. | | | 3.61 | 17.60 | 11.11 |
| E. J. | 6.82 | 7.52 | | | |
| F. B. | 7.00 | 6.27 | 3.13 | 12.82 | 12.82 |
| F. C. | | | 4.85 | 17.79 | 15.20 |
| J. L. | 8.23 | 7.25 | 6.40 | 17.62 | 20.10 |
| F. H. | 7.44 | 8.13 | 5.92 | 23.52 | 25.64 |
| F. B. | | | 5.00 | 20.72 | 20.00 |
| F. C. | 5.40 | 5.83 | 2.87 | 17.16 | 18.77 |
| I. R. | 7.00 | 6.86 | 3.77 | 20.61 | 22.59 |
| F. B. | | | 5.71 | 18.64 | 20.00 |
| O. H. | 5.66 | 6.36 | 3.64 | 16.26 | 16.66 |
| F. B. | 4.84 | 5.18 | 4.83 | 19.23 | 18.86 |
| E. J. | 6.73 | 6.48 | | | |
| A. S. | 7.44 | 6.66 | | | |
| R. T. | 7.25 | 6.36 | 1.61 | 17.77 | 23.80 |
| I. R. | 7.29 | 5.62 | 2.79 | 10.19 | 12.69 |
| J. L. | 6.51 | 6.86 | | 10.90 | 14.80 |

TABLE 2—*Concluded*

| SUBJECT | BLOOD 1 | BLOOD 2 | SWEAT | URINE 1 | URINE 2 |
|---|---|---|---|---|---|
| E. J. | 7.69 | 7.69 | 2.33 | 12.81 | 13.90 |
| F. H. | 6.86 | 7.00 | 1.29 | 12.81 | 15.26 |
| F. B. | 6.36 | 7.00 | | | |
| F. C. | 5.38 | 5.53 | 2.59 | 19.86 | 23.39 |
| T. V. | 7.40 | 7.82 | 3.03 | 16.95 | 19.04 |
| F. C. | 5.88 | 6.76 | 3.85 | 16.06 | 15.00 |
| F. B. | 6.79 | 6.96 | | 13.98 | 15.62 |

In these experiments it is well to bear in mind that the subjects were instructed to come to the laboratory in the morning without breakfast or the partaking of any food or fluids from the time of the meal the previous evening. Upon appearance at the laboratory they were given a test meal consisting of about 20 grams of toast and 250 cc. of tea without cream or sugar. This was done mainly to meet the demands of other experiments, but nevertheless it had the advantages in this experiment of having all the subjects under very much the same dietary conditions.

In table 1 we have 28 determinations of the sweat in which we have recorded the amino-acid nitrogen from a minimum of 1.8 to a maximum of 4.7 mgm. per 100 cc. In table 2 are shown 45 experiments with a range of 1.57 to 4.76 mgm. per 100 cc. This latter represents also the minimum and maximum of our total of 73 experiments.

In table 1 there are 21 experiments where it is possible to compare the two samples of blood. In 8 of these the second sample shows an increase over the first, while in 12 of them there is a decrease and in one no change is indicated. On the contrary in table 2, where 44 experiments are offered for comparison, 33 or 75 per cent of the cases show an increase in the second sample.

In the comparison of the urine in table 1 there are 15 out of a possible 24 experiments where the amino-acid nitrogen is higher in the second sample which is 63 per cent. In table 2 there are 24 out of 35 that show an increase or a little less than 69 per cent. Furthermore, in all the experiments taken together, 66 per cent of the cases show that the amino-acid nitrogen is greater in the second sample.

We are not unduly stressing these comparisons for we are as yet unwilling to say that there is any connection with the factor of sweating. In fact the increase is not significant enough to warrant any conjecture on this point.

In concluding it will be noted that the amino-acid nitrogen concentration of the sweat runs anywhere from one-seventh to as high as one-half of that of the urine. There seems to be no definite ratio.

SUMMARY

1. Amino-acid seems to be constant in normal subjects in amounts varying from 1.57 to 4.76 mgm. per 100 cc. as determined in 73 experiments upon 23 different subjects.

2. Our data practically corroborate the higher readings of Embden and Tachau.

3. There are no correlations indicated in the comparisons of the amino-acid nitrogen of the sweat, urine and blood.

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# THE STATIC AND DYNAMIC EFFORT OF THE HEART DURING EJECTION

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ARGUMENT. If the ventricles contracted isometrically—i.e., without change in volume—the surface areas of the intraventricular pressure curves could be used as an index of the total mechanical energy liberated by each beat. In fact, as Hartree and Hill (1921) have shown, the *tension-time value* bears a constant relation to the heat production in skeletal muscle and is therefore a better index of mechanical activity than the total work performed when the muscle shortens.

But such a study of isometrically contracting mammalian ventricles—even if this were feasible for protracted periods—would not be entirely satisfactory for, as Fenn (1923, 1924) has shown, an afterloaded muscle apparently develops an additional amount of energy over and above that produced when it contracts isometrically. The excess energy so developed varies directly with the work performed and inversely with the duration of the isometric phase which precedes shortening.

The only hope of studying the mechanical effort of the heart more extensively lies in the analysis of the tension-time values of ventricular pressure curves obtained when the heart beats in an afterloaded manner, as it does naturally.

Before it is possible to apply such a method of analysis, it is obviously necessary to have a clear conception of what such values represent. The total area enclosed beneath a curve of this type represents the intensity factor of the potential energy developed in excess of that immediately converted into a kinetic form. It is a much better index of the force developed by the beat than the amplitude alone, because it also takes contour and duration into account. According to Weiss (1913) the tension-time value of such curves measures the *static effort* of the heart as distinct from the *dynamic effort* concerned in expelling blood and performing useful external work.[1] However, such values do not estimate the potential

[1] In spite of its importance, this static factor is disregarded in formulae commonly used for calculating the work of the heart, e.g., in the formulae: $W = QR + \frac{wV^2}{2g}$

energy liberated during any single beat because the volumes of the ventricles are constantly changing and the capacity factor is thereby altered in an undeterminable manner. Furthermore, a comparison of the tension-time values of pressure curves obtained under widely varying conditions of the circulation is of still less value as the diastolic and systolic volumes change in addition.

It is obvious, therefore, that we must discover some way of taking the changing capacity factor into account if the tension-time values of afterloaded ventricular contractions are to be of service in evaluating the mechanical energy of the heart. A number of considerations suggested

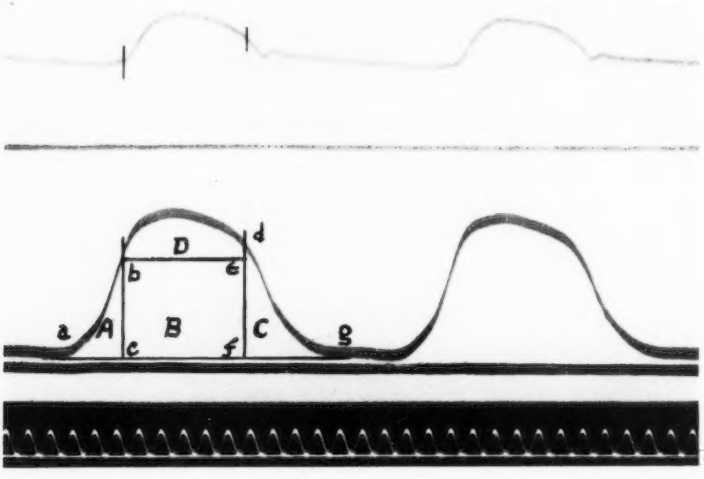



Fig. 1. Curves of aortic pressure (upper) and left ventricular pressure (lower) illustrating method by which onset and end of ejection are determined and manner in which the ventricular pressure curve is divided into areas A, B, C and D. Time 0.02 second. Discussion in text.

that this may be accomplished by subdividing the area bounded by the ventricular pressure curve in accordance with the phases of systole and

(Evans, 1915); $A = pR + \frac{pv^2}{2g}$ (Tigerstedt, 1921); and $A = \int_{P_1}^{P_2} PvD + \frac{Sv^2}{2g}$ (Fahr, 1927). It is, however, taken into account in mathematical expressions which aim to incorporate all the factors involved in the mechanical energy and work of the heart. Thus, we may consider this factor to be included in the fifth term of Frank's equation (1898) for the work of the heart, $A = \int_{V_1}^{V_2} PdV - \int_{V_1}^{V_2} \psi\,(V)dV + \Sigma\frac{dmv_1^2}{2} - \Sigma\frac{dmv_2^2}{2} + R \pm Ai + W$; and Weiss (1913) definitely includes it as a second term in the equation $D = Ph + Qs + Qv$.

diastole. In an intraventricular curve, such as is reproduced in figure 1, the opening of the semilunar valves is indicated at a point *b* synchronous with the rise of aortic pressure. During the interval that the pressure curve rises and until the point *b* is reached, the ventricle contracts isometrically. Area A thus becomes an index of the static effort required to raise the blood from the level existing in the auricle to that obtaining in the aorta. After ejection has stopped at *d* and until inflow begins approximately at *g*, the ventricles relax isometrically. Hence area C represents the intensity of the energy changes involved in reducing the pressure level to that existing in the auricle.[2] It is obvious at a glance that this area is considerably larger than area A, but, as the ventricles are relatively empty during isometric relaxation, the capacity factor is much smaller than during isometric contraction. Hence, a comparison of the two areas is of no value as far as the relative energies involved are concerned, nor are they directly comparable with areas marked out during the ejection phase. This incidentally emphasizes a fact that must be kept in mind in evaluating the changes in any subdivision of the pressure curve, viz., that changes in the tension-time values are indicative of corresponding alterations of the energy developed only when there is evidence that the capacity factor varies in the same direction.

During the ejection phase which extends from *b* to *d*, the heart performs useful external work in forcing blood into the aorta against resistance and in imparting a certain velocity to it. While the heart is doing this work, it must develop potential energy in excess of that necessary to sustain the pressure along a theoretical line *b-e* which marks the diastolic pressure level existing in the aorta. This effort which is represented by Area B is entirely static. The pressure energy developed over and above this, i.e., that represented by Area D, is immediately utilized to overcome the resistance offered by the continually rising arterial pressure and in this sense may be considered as a static effort. This is indeed the conception of Weiss (1913). An essential difference exists, however, between the energy represented by Areas B and D. The former is not converted into a kinetic form; the latter is available for conversion into energy of flow at some subsequent period of the cardiac cycle and potentially represents a dynamic effort. In this sense and for want of more appropriate terms, we shall consider Area B as an index of the static effort and Area D as a criterion of the dynamic effort of the ventricle during the ejection phase. According to this conception Areas B and D are not only measures of the energy available for sustaining the aortic pressure and for conversion into flow but, inasmuch as the ventricular volume changes affect both areas simultaneously and to an equal extent, they also become actual indexes of the

[2] Actually inflow begins a few $\sigma$ before point *g*. This is ignored in our construction as the difference in area is only of academic interest.

potential energy liberated for these respective purposes under different circulatory conditions. Comparison of areas B and D thus offers a valuable method for studying the relative distribution of the static and dynamic energies developed by a ventricle during ejection; in short, their ratios enable us to express the economy with which the pressure energy developed by the heart beat during ejection is utilized.

This research concerns itself with the application of such a method of analysis to the heart working under different circulatory conditions.

TECHNICAL METHODS. The experiments were made on dogs anesthetized with morphine and chloretone. The general technique used in these experiments was the same as that customarily employed in this laboratory.

Several precautions must be observed in recording the intraventricular and aortic pressure curves for this type of analysis. The optical manometers must have a *figure of merit* which is adequate to reproduce the contour, amplitude, and phasic relations of the pressure changes accurately. Errors of parallax must be scrupulously avoided or carefully evaluated. The photosensitive surface on which the records are photographed must move at a uniform speed, preferably not less than 100 mm. per second. Care must be taken that the cannula of the ventricular manometer is not in a *cul-de-sac* and that the transmission of pressure is not impeded by a clot in the cannula of either manometer.

We employed the optical manometers described by Wiggers and Baker (1924) which have a natural frequency of about 200 double vibrations per second under the conditions employed. Parallax was avoided by the use of the double-slit lamp described by Katz and Baker (1924). Tests were made periodically during the course of the experiment to demonstrate that no clots existed in the cannulas. Before accepting any curve as depicting the pressure changes in the ventricle, its contour during the ejection phase was compared with that of the aortic curve. Similarity of contour in the two curves during this time was used as the criterion proving that the ventricular cannula was not occluded during any phase of systole. Records which did not show this similarity were discarded. Checks showed that the speed of the camera was uniform.

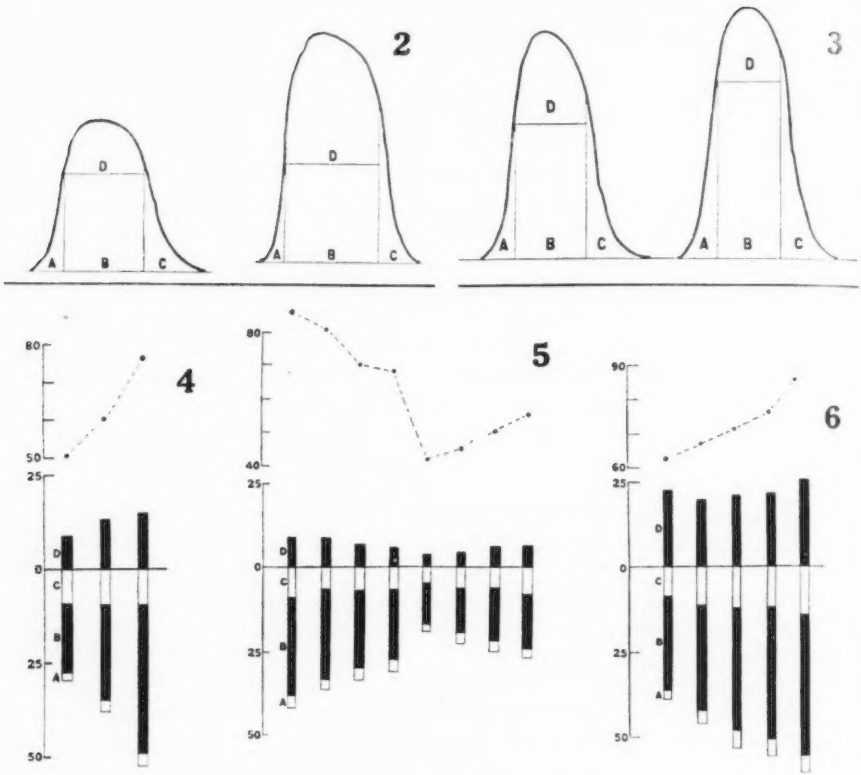
The optically recorded intraventricular pressure curves were enlarged fourfold and accurately redrawn by use of an optical reflectoscope system, the bottom of the pressure line being followed in retracing the curves. During this procedure, the onset and end of ejection were accurately located on the transcribed ventricular pressure curve by reference, respectively, to the primary rise and to the start of the incisura of the aortic pressure curve which was simultaneously projected but not redrawn. A base line was drawn at the bottom of the pressure curve on a level with the pressure existing in the ventricles immediately preceding the onset of systole, i.e., at the initial pressure level. Perpendiculars were then

erected to the points marking the onset and end of ejection, e.g., *b* and *d* in figure 1, and finally a horizontal line, e.g., *b-e* in figure 1, was drawn parallel to the base line at the pressure level existing in the ventricle at the onset of ejection. As already mentioned, these constructions subdivided the area bounded by the pressure curve and its base line into four sections which will be referred to as areas A, B, C and D, respectively (cf. fig. 1). The selection of the points and the construction of the lines were made by the authors personally. The areas were measured with a planimeter and are expressed throughout the paper in sq. cm. While data concerning all the areas are given, emphasis is laid in this paper on areas B and D of the ejection phase.

RESULTS. *The effect of a single variable on the relative size of areas B and D:* In the intact animal, changes in initial stretch of the ventricle or in the arterial resistance rarely occur independently of each other. Very often they are also associated with changes in rate and nutrition of the heart. It is advantageous for several reasons to start our analysis with cases in which only a single variable, i.e., either initial stretch or arterial resistance, is introduced at a time.

Figure 2 shows records from an experiment in which the chief variable was the initial stretch. The curve on the left represents the pressure variations under control conditions, the one on the right, those occurring after saline infusion. The heart rate was kept constant by rhythmic artificial stimulation of the right auricle. The blood pressure and the reaction of the heart attest to the existence of an adequate nutrition. Most of the changes which result from a saline infusion (cf. Wiggers and Katz, 1922) are to be seen or inferred from the curves in figure 2. The change in the level from which the pressure curves rise indicates the increase in initial stretch. The practically constant diastolic pressure which is caused by the balance between the greater cardiac output and the diminished blood viscosity is shown by the constant level of the horizontal line separating areas B and D. The sharper rise of the curve during ejection indicates a greater velocity of ejection. The ordinate value of area D signifies an increased pulse pressure; while the abscissal values of areas A, C and B show the abbreviation of the isometric periods and the prolongation of total systole and of the ejection phase. The application of any work formula would show that the work of the heart is considerably augmented, chiefly because a greater volume of blood is ejected (cf. Evans, 1915, 1918; Straub, 1917; Starling and Visscher, 1927).

An evaluation of the areas of the two curves of figure 2, subdivided in the manner which we have developed, brings out several interesting facts. The tension-time value of the whole curve is increased more than 50 per cent by saline infusion, as is apparent even from a superficial examination of the curves. Not all of the areas share equally in this increase; on the




Figs. 2–6

Fig. 2. Transcribed curves of left ventricular pressures before and after a saline infusion which caused no change in heart rate or diastolic pressure. Left curve, control; right, during infusion. Effects on different areas discussed in text.

DATA

| | ISOMETRIC CONTRACTION | EJECTION | ISOMETRIC RELAXATION | DIASTOLIC PRESSURE |
|---|---|---|---|---|
| | *seconds* | *seconds* | *seconds* | *mm.* Hg |
| Control | 0.054 | 0.134 | 0.086 | 50 |
| After saline infusion | 0.035 | 0.156 | 0.065 | 54 |

Fig. 3. Transcribed left ventricular pressure curves showing changes in various areas following an increased arterial resistance without change in initial tension or heart rate. Left curve, control; right, 4th beat following aortic compression. Discussion in text.

DATA

| | ISOMETRIC CONTRACTION | EJECTION | ISOMETRIC RELAXATION | DIASTOLIC PRESSURE |
|---|---|---|---|---|
| | *second* | *second* | *second* | *mm. Hg* |
| Control | 0.058 | 0.088 | 0.062 | 50 |
| During aortic compression | 0.065 | 0.078 | 0.065 | 94 |

Fig. 4. Chart showing in diagrammatic form size of areas A, B, C and D before (1st block) and during saline infusion. Ordinate values in sq. cm. Black rectangles show ejection areas B and D; white rectangles, the isometric areas, A and C. Upper plot, diastolic pressures in mm. Hg. Discussion in text.

DATA

| | ISOMETRIC CONTRACTION | EJECTION | ISOMETRIC RELAXATION | RATIO AREAS D:B |
|---|---|---|---|---|
| | *second* | *second* | *second* | |
| Control | 0.050 | 0.100 | 0.085 | 0.462 |
| During saline infusion | 0.045 | 0.112 | 0.125 | 0.515 |
| | 0.050 | 0.112 | 0.123 | 0.374 |

Fig. 5. Chart showing effects of vena cava compression (2nd to 5th block, incl.) and decompression (6th block, etc.) on areas A, B, C and D. Ordinates, sq. cm. Upper plot, diastolic pressure changes in mm. Hg. Discussion in text.

DATA OF SUCCESSIVE PLOTS

| ISOMETRIC CONTRACTION | EJECTION | ISOMETRIC RELAXATION | RATIO AREAS D:B |
|---|---|---|---|
| *second* | *second* | *second* | |
| 0.052 | 0.115 | 0.067 | 0.313 |
| 0.050 | 0.116 | 0.066 | 0.340 |
| 0.052 | 0.107 | 0.078 | 0.302 |
| 0.054 | 0.100 | 0.085 | 0.293 |
| 0.030 | 0.084 | 0.100 | 0.368 |
| 0.047 | 0.092 | 0.092 | 0.364 |
| 0.045 | 0.100 | 0.084 | 0.437 |
| 0.043 | 0.100 | 0.080 | 0.427 |

Fig. 6. Chart showing effects of compressing thoracic aorta on areas A, B, C and D. In order: control beat, beats 1, 2, 6 and 10 after compression. Ordinates, sq. cm. Upper plot, diastolic pressure in mm. Hg.

DATA OF SUCCESSIVE PLOTS

| ISOMETRIC CONTRACTION | EJECTION | ISOMETRIC RELAXATION | RATIO AREAS D:B |
|---|---|---|---|
| *second* | *second* | *second* | |
| 0.054 | 0.130 | 0.104 | 0.850 |
| 0.053 | 0.126 | 0.112 | 0.642 |
| 0.050 | 0.129 | 0.100 | 0.590 |
| 0.059 | 0.129 | 0.110 | 0.580 |
| 0.056 | 0.130 | 0.124 | 0.620 |

contrary, areas A and C decrease. The decrease in these areas is caused by the steeper gradient of the curve and the abbreviation of the isometric contraction and relaxation periods. The higher pressure level from which the curve starts to fall during isometric relaxation minimizes the change in area C. It may be reëmphasized that these changes do not necessarily imply that the mechanical energy developed during these phases is also lessened; the volume of the ventricular chamber is undoubtedly larger.

The increase in the tension-time value of the curve occurs entirely during ejection. This is significant since the capacity factor is also increased during this period, owing to the larger size of the ventricle and its greater systolic discharge. Incidentally, it is interesting to note that, as in skeletal muscle, this increased mobilization of energy during ejection is associated with an abbreviation of the preceding phase of isometric contraction. Even a superficial examination shows that area D profits most by the increase in mechanical energy. All three factors—amplitude, duration, and contour—contribute to the increase in this area, but the first factor is the most important. The smaller increase in area B is due to the prolongation of the ejection time and occurs in spite of the slight diminution in its ordinate value.

Calculations show that the ratio $\frac{\text{area B}}{\text{area D}}$ increases markedly, namely, from $\frac{41.8}{100}$ to $\frac{112}{100}$ or, stated in another way, the energy capable of being utilized for dynamic purposes increases from 29.5 per cent to 52.8 per cent of that developed during ejection.

These results indicate that an increase in the initial stretch of the heart which is not excessive and is not associated with any increase in arterial resistance leads to a more efficient distribution of the energy in that a greater proportion of the total energy is available for dynamic purposes.

In figure 3, an experiment is represented in which the chief variable is the arterial resistance. The heart rate was kept constant in the same way as in the previous experiment. The curve to the left is a beat recorded under control conditions, the one to the right, the fourth beat following compression of the aorta. This figure shows the changes which occur after an increase in arterial resistance before compensatory changes due to the secondary increase in initial stretch set in. Most analyses of the effect have been made after these compensatory changes have occurred. In this laboratory, however, the immediate changes following an increase in arterial resistance have been established (Wiggers and Katz, 1922). The typical changes are shown in figure 3. The constancy of the level from which the pressure rises indicates that the initial stretch is unchanged. The higher level of the horizontal line separating areas B and D establishes the higher diastolic pressure; the smaller ordinate value of area D indicates

the decrease in pulse pressure; the abscissal values of areas A and B indicate a slight prolongation of the isometric contraction phase and an abbreviation of total systole and of the ejection phase. It has previously been shown that the systolic discharge is smaller (Wiggers and Katz, 1922).

Measurement of the area of the two curves shows that an augmentation in arterial resistance causes an increase (of about 15 per cent) which is surprisingly small when the rise of the diastolic pressure level is considered. More surprising still, the combined area of ejection (area B and D) decreases slightly. In order to realize the importance of these changes, we must briefly state the probable conditions obtaining. The total mechanical energy liberated is actually not less than normal but is equal to or even greater than normal. The velocity with which blood is ejected is decreased and on this account the kinetic energy is lessened. The higher diastolic pressure requires that a greater proportion of the pressure energy be expended in overcoming and sustaining the greater arterial resistance. The increase in the isometric areas A and C and that of the sustaining area B mirror these changes.

The increase in areas A and C is due to the augmented difference of levels between which the pressure changes during these phases, and in the former also to the prolongation of the phase of isometric contraction. The increase in area B is due entirely to the higher level at which the pressure is sustained and occurs despite the abbreviation of ejection time. The marked reduction in area D is the combined effect of a decrease in amplitude and in ejection time.

It is apparent from a comparison of the relative sizes of areas B and D that the economy of the distribution of the mechanical energy developed during ejection is lessened considerably. Calculations of the ratio $\frac{\text{area D}}{\text{area B}}$ show that it decreases from $\frac{54.4}{100}$ to $\frac{22}{100}$ or, stated in other words, the percentage of energy developed during ejection capable of being utilized for dynamic purposes decreases from 35.2 per cent to 18.0 per cent.

Such results do not support the idea that a greater afterload has a beneficial influence either in increasing the total force of the beat or in effecting a more economical utilization of the pressure energy. It is conclusively shown that the economy of the distribution is markedly diminished.

*The interacting effects of various degrees of initial stretch and arterial resistance on the relative size of areas B and D.* As already indicated, the simple effect of an increased initial stretch or of an increased arterial resistance is rarely encountered in the intact animal or in man. Usually when circulatory conditions change, both factors alter. The group of experiments now to be described shows the effect of various degrees of initial stretch and

arterial resistance on the relative sizes of the areas. The heart rate was constant in these experiments, as was also the alveolar $CO_2$ tension.

In order to present analyses made under these more complex circulatory conditions in a concise form, charts were made of the data obtained from the enlarged redrawn curves. The discussion which follows is based on such charts as are illustrated in figure 4 *et seq.* In the charts, a rectangle is plotted above the zero line so that its *height* represents the tension-time value of area D, as indicated on the scale to the left. Rectangles are likewise plotted below the line to show by their lengths the comparative size of areas A, B and C. The larger black rectangles representing areas B and D are especially stressed. The durations of the various phases are given in the legends. In addition, the diastolic pressures are plotted in the upper part of the chart.

*a. The effect of variations in venous return.* Saline infusion does not always lower the viscosity of the blood sufficiently to maintain a constant arterial resistance, as in the experiment illustrated in figure 2. On the contrary, a considerable rise in arterial resistance frequently accompanies the saline infusion especially when rapidly given. This combination is comparable with that which occurs in the unoperated animal when the venous return is augmented and the heart rate remains unchanged. The effect of such a combination following saline infusion is shown in figure 4. The rectangle to the left gives the areas of the control beat and the two succeeding ones, those of beats during two stages of infusion. The elevation of diastolic pressure is graphically shown in the upper plot, and the prolongation of ejection is recorded in the data in the legend.

The total area of the pressure curve increases during the infusion, but the relative sizes of the various subdivisions are different from those noticed when the arterial resistance remained constant. The isometric areas A and C increase slightly, whereas in figure 2 they decrease. In both cases, the effect on area A is most marked. The increase in the isometric areas in figure 4 is attributable to the steeper gradient and to the greater pressure change during these periods. In this respect, the increase is similar to that occurring when the arterial resistance is augmented without change in initial stretch (cf. fig. 3). The combined area of ejection (area B plus D) is also increased by saline infusion, but, unlike figure 2, the increase is shared nearly proportionally by the two areas; the ratio $\frac{\text{area D}}{\text{area B}}$ being 0.462 in the control and 0.515 and 0.374, respectively, in the two stages of infusion. It follows that the augmentation of arterial resistance counterbalances the effect of the increased initial stretch so that the efficiency with which the greater pressure energy[3] is utilized for static and dynamic purposes during ejection is not appreciably altered.

[3] Such an increase in pressure energy is inferred because the capacity factor also increased.

While saline infusion usually increases the total tension-time value of the pressure curve, continuation of the infusion, particularly if it is rapid, sooner or later leads to a reduction which may ultimately make the tension-time value smaller than normal. It was found that, when such a decrease occurs, the distribution of the pressure energy is more economical; a greater proportion becomes available for dynamic purposes. Thus, in one experiment the ratio $\frac{\text{area D}}{\text{area B}}$ increased from 0.493 in the stage of infusion in which the total area increased to 1.70 and 1.80 in stages in which the total area decreased. This remarkable effect of overdistention is attributable to the lowering of the diastolic arterial pressure and to the greater initial stretch, the latter causing area B to decrease and area D to change but little.

The fall in arterial resistance which accompanies a marked reduction in venous return as, for example, that caused by compression of the inferior vena cava is constant. Even complete compression of the aorta does not counterbalance such a fall in arterial pressure. In this respect, the effect is typical of the changes which occur in shock and hemorrhage. The typical effect of vena cava compression on the tension-time values is shown in figure 5. The rectangle at the left represents the data of the control beat; the next three, the data of the first three beats following the application of the compressor; the fifth block, the data of a subsequent stabilized beat; and the last three, the data of the first three beats following release of the compression.

Analysis of the records shows that such compression causes a decrease in systolic discharge, in the blood pressures, in pulse pressure, in the duration of ejection and total systole, and, after a time, in the isometric contraction phase.

The total area becomes progressively smaller during the compression, a decrease that affects all of the areas. The reduction in areas B and D is proportional, as shown by the fact that the ratio $\frac{\text{area D}}{\text{area B}}$ changes little, i.e., from 0.313 in the control to 0.34, 0.302, 0.293, 0.368, respectively, in the next four beats given in figure 5.

When the compression is released, conditions do not at once return to normal. The low arterial resistance and the abbreviation of the systolic phases persist. While the total area increases somewhat, it is still below the control value even in the third beat. The distribution of the pressure energy during ejection becomes more efficient, the ratios $\frac{\text{area D}}{\text{area B}}$ are 0.364, 0.437 and 0.427, respectively, in the last three beats of figure 5, as compared with the value of 0.313 in the control beat. Similar changes were observed in this animal when vena cava compression was produced

during the pressor action of central vagus stimulation, except, of course, that the total tension-time values were greater and the ratios $\frac{\text{area D}}{\text{area B}}$ smaller on account of the larger arterial resistance.

The results given above indicate that, when the venous return is altered, the change in initial stretch is counterbalanced by the secondary change in arterial afterload, so that the efficiency with which energy is distributed during ejection tends to remain the same.

*b. The effect of variations in arterial resistance.* It is interesting to see whether or not a similar state of events also occurs when arterial resistance is the primary variable. In the experiments to be described, the changes in arterial resistance were produced either by digital compression of the lower thoracic or abdominal aorta or by the pressor and depressor action resulting from stimulation of the central end of the cut vagus nerve. Such experiments are probably indicative of the economy with which the heart ejects its blood in clinical cases of hyper- and hypo-tension.

Except for the first few beats, the effect of the change in arterial resistance is complicated by alterations in initial stretch. These result from the variable retention of blood within the ventricle. This is particularly noticeable when the aorta is mechanically compressed because the capacity of the arteries in free communication with the ventricles is decreased at once. The results of an aortic compression experiment are shown in figure 6. The rectangle to the left gives the areas of the control beat and the next four, the area of the 1st, 2nd, 6th and 10th beats following aortic compression. The progressive rise in diastolic pressure is shown on the graph above. The early abbreviation of ejection which returns to the control value in the 3rd plotted beat is shown by data in the legend. Measurements of the level from which the pressure curve rose indicate that the initial stretch of the ventricle increased progressively.

The total area becomes progressively larger during the compression, as do also the isometric areas A and C and the ejection area B. The combined area of ejection (B plus D) decreases in the first beat, as in figure 2, but, in the beat following, it becomes larger than normal and thereafter increases progressively. Similar changes occur in area D except that it does not exceed the control value until the 5th beat plotted.[4]

The changes in the ejection areas are not to be taken as indicating like changes in the pressure energy developed, for the capacity factor is undeterminable and a prediction would be no more than a guess. Nevertheless, the results show that the uneconomical distribution of the pressure energy developed during ejection tends to be improved slightly as stable

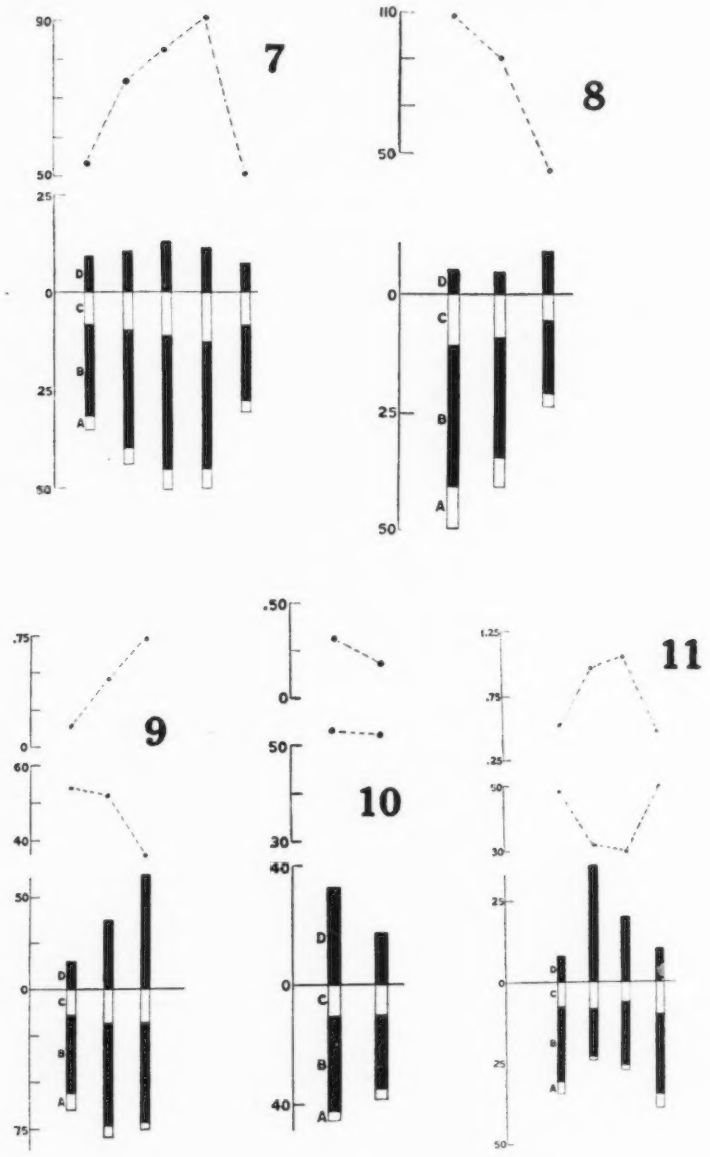
[4] The increase in area D which occurs without much change in amplitude and duration is caused by the alterations in the contour of the arch-shaped summit.

conditions are reached. This is shown by the values of the ratio $\frac{\text{area D}}{\text{area B}}$ which is 0.85 in the control and 0.642, 0.59, 0.58, 0.62, respectively, in the other beats shown in figure 6. In other experiments in which beats, recorded after stabilized conditions had been established, were compared with the control beats, the secondary influence of initial stretch was usually greater. In some experiments, it was sufficient to counterbalance the detrimental effect of the augmented afterload so that the ratio $\frac{\text{area D}}{\text{area B}}$ was the same after compression as before. For example, in experiment C-375, calculations showed that the ratio was 0.877 in both beats. Usually, however, the deleterious effect of the increased afterload predominated. We have found no instance in which the effect of the secondary increase in initial stretch predominated. It follows that the maintenance of the same minute-output is accomplished less economically when the arterial resistance is high. In other words, the heart has to expend more mechanical energy under these conditions in order to discharge a constant amount of blood. This statment is based on the fact that the capacity factor of discharge is assumed to be constant under these conditions while the volume of the heart is larger.

When the central end of a vagus nerve is stimulated, the steps are not so well demarcated because the effect of the initial stretch tends to fuse more gradually with the effect of the arterial resistance. This is partly due to the constriction of the splanchnic vessels which seems to augment the venous return in the early stages. Fundamentally, the results are the same as with aortic compression.

Figure 7 gives the data of such a central vagus pressor action. The rectangle on the left gives the data of the control beat; the three succeeding ones show the data of the 15th, 30th and 45th beat during stimulation, and the last block shows the data of a beat some time after stimulation had been stopped. The rise in diastolic pressure is shown in the graph above and the anticipated abbreviation of the ejection phase is recorded in the legend.

The total area of the curve increases during the pressor action. This increase is shared by all of the areas. The economy with which the energy is utilized during ejection is decreased slightly, as indicated by the diminution of the ratio $\frac{\text{area D}}{\text{area B}}$ from 0.392 in the control to 0.342, 0.374 and 0.358 in the beats during stimulation. The response is not constant, however. For example, in another pressor experiment, made in the same animal shortly after the one described, the ratio $\frac{\text{area D}}{\text{area B}}$ increased slightly from 0.372 in the control to 0.41 and 0.396 in the 15th and 30th beats during stimulation; in the 45th beat, the ratio had decreased even below the




Figs. 7–11

Fig. 7. Chart showing effects of central vagus stimulation (pressor effect) on areas A, B, C and D. In order: control beat, 15th, 30th and 45th beat during stimulation, and beat after recovery. Ordinates, sq. cm. Upper plot, diastolic pressure in mm. Hg.

DATA OF SUCCESSIVE PLOTS

| ISOMETRIC CONTRACTION | EJECTION | ISOMETRIC RELAXATION | RATIO AREAS D:B |
|---|---|---|---|
| *second* | *second* | *second* | |
| 0.060 | 0.120 | 0.109 | 0.392 |
| 0.057 | 0.111 | 0.101 | 0.342 |
| 0.065 | 0.114 | 0.108 | 0.374 |
| 0.052 | 0.117 | 0.105 | 0.358 |
| 0.055 | 0.115 | 0.100 | 0.375 |

Fig. 8. Chart showing effects of central vagal depressor reaction on areas A, B, C and D. Ordinates, sq. cm. Upper plot, diastolic pressure in mm. Hg.

DATA OF SUCCESSIVE PLOTS

| ISOMETRIC CONTRACTION | EJECTION | ISOMETRIC RELAXATION | RATIO AREAS D:B |
|---|---|---|---|
| *second* | *second* | *second* | |
| 0.080 | 0.098 | 0.085 | 0.171 |
| 0.068 | 0.102 | 0.085 | 0.188 |
| 0.065 | 0.104 | 0.096 | 0.504 |

Fig. 9. Chart showing effect of cardiac slowing by vagal stimulation on areas A, B, C and D. Ordinates, sq. cm. Upper plot, durations of previous diastoles in sec., middle plot, diastolic pressure in mm. Hg.

DATA OF PLOTTED BEATS

| ISOMETRIC CONTRACTION | EJECTION | ISOMETRIC RELAXATION | RATIO AREAS D:B |
|---|---|---|---|
| *second* | *second* | *second* | |
| 0.076 | 0.102 | 0.109 | 0.375 |
| 0.070 | 0.145 | 0.109 | 0.655 |
| 0.050 | 0.170 | 0.111 | 1.160 |

Fig. 10. Chart showing effect of artificial acceleration of heart on areas A, B, C and D. Ordinates, sq. cm. Upper plot, durations of previous diastoles in sec., middle plot, diastolic pressure in mm. Hg.

DATA OF PLOTTED BEATS

| | ISOMETRIC CONTRACTION | EJECTION | ISOMETRIC RELAXATION | RATIO AREAS D:B |
|---|---|---|---|---|
| | *second* | *second* | *second* | |
| Natural slow rate | 0.072 | 0.200 | 0.148 | 0.71 |
| Artificial rapid rate | 0.069 | 0.150 | 0.160 | 1.05 |

Fig. 11. Chart showing effect of persistent vagal slowing on areas A, B, C and D. In order: normal control, immediate effect of slowing, prolonged slowing, and after recovery. Ordinates ,sq. cm. Upper plot, durations of previous diastoles in sec., middle plot, diastolic pressure in mm. Hg.

DATA IN SUCCESSIVE PLOTS

| ISOMETRIC CONTRACTION | EJECTION | ISOMETRIC RELAXATION | RATIO AREAS D:B |
|---|---|---|---|
| *second* | *second* | *second* | |
| 0.066 | 0.134 | 0.138 | 0.362 |
| 0.038 | 0.202 | 0.130 | 2.440 |
| 0.075 | 0.200 | 0.129 | 1.040 |
| 0.074 | 0.142 | 0.130 | 0.440 |

control, i.e., to 0.278. This variability is understandable when all of the possible combinations of initial stretch and arterial afterload are taken into account.

Conditions do not return at once to normal after the pressor action is over. Thus, in figure 7, the abbreviation of ejection persists. The area of the curve remains below that of the control on account of the decrease in the value of the combined area of ejection (B plus D). The ratio $\frac{\text{area D}}{\text{area B}}$ changes very little however, i.e., from 0.392 in the control to 0.375 in the beat after stimulation had ceased.

When stimulation of the central end of the vagus nerve causes a depressor effect, the results are the reverse of those obtained with a pressor action. In figure 8, the data of such an experiment are shown. The rectangle on the left represents the data of the control beat, the next two show data of the two beats during the depressor action. The arterial resistance was high at the start. The fall in diastolic pressure, the prolongation of ejection, and the abbreviation of the isometric contraction phase are shown in the chart and its legend. The total area of the curve decreases, a decrease that is shared by the isometric areas A and C and the combined area of ejection (B plus D). Area D at first decreases but in the later stage becomes larger than in the control. The ratio $\frac{\text{area D}}{\text{area B}}$ increases slightly at first from 0.171 to 0.188 and then markedly to 0.504 in the last stage of the depressor action.

The observations described following the depressor action show that the heart responds in the anticipated way when the blood pressure falls, even though some impairment of coronary flow must occur. No determinations were made of the critical level beyond which nutritional influences are introduced.

*The effect of variations in heart rate on the relative size of areas B and D.*

Up to the present, we have considered only the effects of changing venous return and arterial resistance when the heart rate is constant. Heart rate changes cannot be ignored, however; on the contrary, they are more important than any other factor in compensating for alterations in the circulatory conditions.

In the experiments to be described, the heart rate was slowed by stimulation of the peripheral end of the vagus nerve and accelerated by applying artificial stimuli to the right auricle at various rates. No attempt was made to keep the blood pressure constant, and, in all but the last experiment to be described, nutritional impairment could be excluded. Hence, the results probably resemble those occurring in the unoperated animal or in man. The range of heart rates analyzed varied from 250 to 48 beats per minute. The graphs were made in the same way as in the preceding figures, but contain, in addition, a curve showing the durations of previous diastole.

The typical effect following moderate vagus stimulation is shown in figure 9. The rectangles from left to right give, respectively, data of the control and of two successive beats early during stimulation. Such slowing produces a fall in diastolic pressure and a greater diastolic filling, both of which should on a priori grounds favor a more economical use of the energy during ejection. The results show that this is the case. The increase in the total area during slower beats affects mainly area D. The slight decrease in area A is caused by the abbreviation of the isometric contraction phase and by the decrease between the pressure level in the aorta and in the left auricle. Area C increases slightly due to an increase in the pressure level existing in the aorta at the end of ejection. Area B changes very little as a result of a nice balance between the opposing action of the prolongation of the ejection time and the lower arterial diastolic pressure level. Examination of the original curves shows that the large increase in area D is caused not only by the prolongation of ejection and the greater height of this area but also to a large extent by the change in contour of the arch-shaped summit. It is apparent from these observations that the economy of energy utilization during ejection is considerably increased by slowing the heart, e.g., in the experiment shown in figure 9, the ratio $\frac{\text{area D}}{\text{area B}}$ is increased from 0.375 in the control to 0.655 and 1.16 after slowing. In other words, a slower heart rate leads to a better utilization of the pressure energy which is developed.

Under natural conditions, the fall in diastolic pressure which accompanies slowing of the heart is usually counterbalanced by compensatory mechanisms, and, in such cases, the effect of the increased initial stretch only is concerned. This is illustrated by results shown in figure 10. The rectangle to the left gives the data of the slower beat which was determined by

the rate of the normal pacemaker; the one on the right gives the data of a beat taken some time after the establishment of a faster artificial rhythm through the right auricle. In this experiment, the peripheral compensatory mechanisms kept the diastolic arterial pressure constant. If, for the sake of comparison with figure 9, we consider the faster rate as the control, it is observed that the changes in tension-time value of the whole curve and its subdivision are the same except that area B increases somewhat more than area D. The improvement in the economy with which the blood is ejected in the slower beat is, therefore, not so great. For example, in figure 10, the ratio $\frac{\text{area D}}{\text{area B}}$ is 0.71 in the rapid and 1.05 in the slow beat.

The increase in area B and D combined indicates an actual augmentation of the pressure energy developed during ejection as the capacity factor is also increased. The constancy of area B in figure 9, however, does not preclude such an increase. It follows from the results given above that the maintenance of a constant minute-output can be accomplished much more economically at a slow rate than at a rapid one.

When a very slow rate and a low blood pressure are maintained for some time, the nutrition of the heart is impaired and certain interesting changes are observed. The results of such an experiment are shown in figure 11. The rectangle on the left gives the data of the control beat, the second one, the data of a beat recorded early during vagus slowing, the third, that of a beat recorded after slowing had been maintained for some time, and the last block gives the data of a beat when the circulation had practically returned to control conditions.

The differences between the two left hand blocks are typical (compare with fig. 9) except that area B decreases because the effect of the lower diastolic pressure predominates. A comparison of the middle two blocks shows the effect of impairment of nutrition, the rate of the heart and the diastolic pressure being practically the same in both, as is also the duration of ejection. The impairment in nutrition decreases the total area of the pressure curve, a decrease which affects only areas C and D. The decrease in area D is caused by the smaller amplitude and especially by the more peaked character of the summit. The decrease in area C is caused by the fall in the pressure level existing in the ventricle at the end of ejection. Areas A and B increase slightly, the former because the period of isometric contraction is prolonged and the latter because the initial pressure is decreased. The redistribution of the energy developed during ejection is strikingly more inefficient when the cardiac nutrition is impaired, e.g., the ratio $\frac{\text{area D}}{\text{area B}}$ decreases from 2.44 to 1.04, nevertheless it is still much larger than the faster control beat in which the ratio is 0.362.

Discussion. An analysis of the expenditure of energy during the ejec-

tion phase has established a number of facts, the significance of which merits brief discussion.

Our results emphasize the fact that the initial stretch of the ventricular muscle not only governs the volume of systolic discharge (which is well established) but also regulates the economy with which the pressure energy is utilized. This discloses a hitherto unrecognized "factor of safety," the importance of which in safeguarding the heart against fatigue and in maintaining a greater margin of so-called *cardiac reserve* cannot be too highly stressed.

This factor operates to conserve the energy of the heart, not only when called upon to increase its systolic discharge as a result of a greater venous return, but also when changes in heart rate or arterial resistance occur primarily.

It is generally recognized that a rapid action of the heart may be detrimental, partly because it fails as a pump to maintain an adequate pressure in the arteries and partly because the rapid rate *per se* tends to produce fatigue and cardiac failure. On the other hand, the advantage of a slow heart rate in the athlete and the beneficial effects derived from keeping the heart action slow in cases of impending failure are well known. The relative advantage of a slow over a rapid heart has been attributed somewhat vaguely to the theoretical benefit of the diastolic rest period. Our analyses indicate that a different mechanism is chiefly concerned. When the heart rate is slow, the interval of diastolic filling is prolonged, the initial stretch is increased, and the pressure energy developed during ejection is utilized to a greater extent for dynamic purposes just as is the case when the initial stretch is increased at constant heart rates by an increased venous return. Likewise, the smaller percentage of the mechanical effort available for dynamic purposes when the heart rate is rapid corresponds to the effect of reducing the venous return when the heart rate is kept constant. It is readily seen, therefore, that the same minute-output is attained more economically at slow rates.

An increased arterial resistance, such as the heart is required to work against in clinical hypertension, is shown to be without any stimulating effect on the ventricle, indeed, a larger proportion of the energy developed during ejection is needed to sustain the pressure at the higher level and a smaller portion is left for dynamic purposes. This is particularly significant in view of the fact that the greater work of the heart in hypertension is determined, according to Fahr (1927), by the systolic arterial pressure. In other words, an increased arterial load augments the work of the left ventricle but in itself supplies only a mechanism which causes the heart to work in a less economical manner. Early fatigue and failure under such conditions are avoided only because a mechanism is provided whereby the initial stretch is at once increased. This, as our results indicate, operates

to reëstablish the normal relation between the energy available for static and dynamic purposes.

One other fact may be emphasized. It was pointed out in considering the effect of heart rate changes that the increase in the pressure area D was not entirely due to the greater duration of ejection nor to the larger pulse pressure, but in part, at least, to the changes in contour of its summit. These observations clearly emphasize that duration and contour play as great a rôle as amplitude in determining the vigor and economy of the beat. Heretofore, it has been impossible in pharmacological and physiological investigations to do more than take the amplitude changes into account in estimating vigor. This has been obviated by our method of analysis which incorporates all three factors.

## SUMMARY

*Method.* The method of studying muscular contraction by measuring the area bounded by the tension curves, i.e., by determining their *tension-time* values, cannot be applied to the normal intraventricular pressure curves because the volumes of the ventricles are constantly changing. A method is reported by which this capacity factor is taken into account and is applied to intraventricular pressures recorded from intact animals under a variety of circulatory conditions. An accurately recorded pressure curve is enlarged fourfold and divided into four areas, labeled *A*, *B*, *C* and *D* as shown in figures 1, 2 and 3. Area A is an index of the static effort expended during isometric contraction to raise the blood from a pressure level existing in the left auricle to that obtaining in the aorta. Area C represents the static effort employed during isometric relaxation to lower the pressure to a level existing in the auricle. The changes in these areas under various conditions are incidentally discussed, but especial consideration is given to areas B and D which are marked off under that portion of the curve representing systolic ejection. We consider the lower area (B) to be a measure of the *static effort* of the ventricle during ejection in the sense that it represents the pressure energy required to sustain the blood column at a diastolic level. We regard the upper area (D) as an index of the *dynamic effort* during ejection in the sense that this energy becomes available for conversion into kinetic energy of flow at some subsequent period of the cardiac cycle. A comparison of these two areas (e.g., as a simple ratio, D:B) gives an estimate of the economy with which the pressure energy is utilized during ejection. Such a comparison is permissible since changes in ventricular volume affect both areas simultaneously and equally during successive moments of ejection.

*Results.* 1. When the rate of the heart and the arterial resistance are controlled and are constant, an increase in initial stretch such as follows an augmented venous return increases the tension-time values of areas

B and D combined. This is caused by several factors: the prolongation of the ejection phase, the greater amplitude of the arched summit, and the changes in its contour. The increase in area D is greater than in area B, showing that a larger percent of the extra energy developed during the ejection phase is utilized for dynamic purposes.

2. When the heart rate and initial stretch remain constant, an increased arterial resistance diminishes the size of area B and D combined. This is caused chiefly by the abbreviation of the ejection phase. The ratio area D to area B decreases markedly indicating that a greater portion of the total pressure energy developed during ejection is utilized in the *static effort* in maintaining the pressure at a higher diastolic level, while less is left available for dynamic purposes. An increased arterial resistance is thus shown to be without stimulating action upon the ventricle; it augments the work of the heart but supplies no mechanism which operates to economize the energy of the heart beat.

3. When an increase in arterial resistance occurs in the intact animal, a mechanism is provided which restores the distribution of energy during ejection to normal. The increased resistance to expulsion of blood results in a partial retention during each successive systole and this soon acts to increase the initial stretch. As already analyzed, this change in initial stretch increases the total pressure energy developed during ejection to the advantage of that portion available for dynamic purposes. Consequently, the effects of increased arterial resistance and increased initial stretch either neutralize each other or the effect of the latter predominates. This constitutes the "factor of safety" which safeguards the ventricular muscle against fatigue and restores the margin of so-called *cardiac reserve* to normal.

4. The economy with which the pressure energy during ejection is distributed between areas B and D when the heart beats at different rates also depends on the diastolic arterial pressure and on the initial stretch of the ventricle. But in this case, the two factors affect the economy with which energy is utilized during ejection in the same direction. Thus at slow rates, the diastolic pressure level falls and the initial stretch increases. The size of area B and D combined increases to the advantage of area D. This change is due predominately to the increase in initial stretch. These observations explain why the slowly beating heart is spared and the rapidly beating one, more prone to fatigue.

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# NORMAL RECTAL TEMPERATURES OF SHEEP

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In the study of the effects of poisonous plants on animals many clinical observations, including those on temperature, pulse and respiration, have been taken, not only during the time of the experimental feeding but for variable periods, both before and after the period of feeding and any illness which might result. As a rule such observations have been taken twice daily. The fact that there is very little published information on these data on normal animals which was applicable to the conditions under which this work was done, as well as the desirability of having exact knowledge of the individual animals used in the experiments have been reasons for taking such observations. In this way there has accumulated a considerable amount of evidence regarding normal conditions. In this paper the results in regard to the normal temperature of sheep are gathered together and summarized.

Since 1915 the experimental work on poisonous plants has been carried on in the Wasatch Mountains of South Central Utah on the Fishlake National Forest where a station was established by the Bureau of Animal Industry in coöperation with the Forest Service. This station is near Salina, Utah, and at an altitude of approximately 8,000 feet above sea level. The country about the station is fairly typical of the mountain ranges of that region used for summer pasturage for sheep, cattle and horses. All of the temperature records used in the following paper were obtained at this station, during the months of June, July, August and September, and between the years 1915 and 1926 inclusive.

*Method of handling the sheep.* It has been customary to obtain approximately 50 normal sheep of local owners each spring, take them to the station about the first of June or as soon thereafter as the feed in the pastures is sufficiently far advanced to supply them with an abundance of forage. Each animal is given a number and a record made of its age, sex and weight. These animals are then used as a supply from which individuals are selected for experimental purposes. When not being used

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experimentally they are given the run of the pasture during the day but are kept in corrals at night. Feed, water and salt are available.

When sheep are needed for experimental purposes, they are selected from the band, weighed and put in pens, approximately 10 feet square, where they are fed alfalfa hay and sometimes bran. Each afternoon while being kept in they are allowed to run for about an hour in a larger corral where they have access to water and salt. One-half of the pens are covered over by a roof, the others, while not covered, are more or less shaded by trees.

The observations are started, as a rule, on the morning when the sheep are weighed and put in the small pens. Thus the animals have been in the large corral over night and in the pasture the day previous. As a rule, about 15 hours elapse between the time any one of the sheep is brought in from the pasture and the time the first temperature observations are made. From this time on observations are made, usually twice a day, as long as the animals are kept in the pens. After being under observation for a period the animals may be used for experimental purposes, and if alive at the conclusion of the experiment returned to the pasture. After being kept with the reserve band for a period, usually three weeks or more, such animals as have fully recovered from an experiment or have been uninjured by it are often kept in for a second or even a third or fourth period, and observations made in the same manner as when kept in for the first period. In the following paper only temperature observations taken before the animals have been fed the material being tested and when they were considered normal were used. No observation taken after the sheep had been fed, or given a chance to eat any plant material other than hay or grain was used, excepting after they had been given a sufficient period to recover from any injurious effect. Observations on lambs whose mothers had been given any questionable material have been excluded.

As stated above sheep have been obtained each spring from local owners. For several successive years they were obtained from the same man so that a few individuals were at the station two or more different seasons. Even during this period, however, the great bulk of the animals were individuals which were at the station only the one season.

The sheep brought to the station each spring have been fairly representative of the bands from which they came. In selecting them naturally the best animals have not been taken, nor animals not considered normal. As a rule, comparatively few ewes with lambs have been used. None of the sheep were pure-bred, but the predominating blood has been Rambouillet. A few showing Cotswold or Lincoln blood have been included.

It has been customary to employ two or three young men at the station each summer, one of whose duties has been the taking of routine tempera-

ture observations on the experimental animals. This has, however, been under the immediate supervision of experienced people and the observations frequently checked. Thus the records are the accumulated results of observations made by a considerable number of people. The temperature records are all taken from the rectum with four inch clinical thermometers. As these thermometers have, as a rule, had a total length of very little over four inches they have for the most part been inserted between three and four inches into the rectum. This is probably of some importance as Hobday (1896) found that "The distance to which the thermometer is inserted makes a very appreciable difference, sometimes even as much as a degree." Hobday compared four inch thermometers with others which were five or six inches long.

There were obtained in this way 5,826 observations on 514 different normal sheep. In an attempt to analyze the effects of various factors these, as will be seen, were grouped in different ways. The observations on a few individuals could not be used in certain of the groups because of incomplete records as to age or sex.

*Treatment of data.* The individual records obtained have been treated by the ordinary biometric method as described and discussed by Davenport (1914) and Pearl (1923). For most of the constants the shorter methods as given by Pearl, pp. 264 to 279, have been followed very closely. The temperature observations were arranged in the form of frequency tables. While the temperature observations when taken were recorded to the nearest tenth of a degree Fahrenheit, for treatment they were arranged in groups, each group including 6 of the $\frac{1}{10}$ degree, the range between the lower and upper limits of each group being $\frac{6}{10}$ of a degree. In this way 15 temperature classes were obtained. The frequency distribution table obtained in this manner for the 8,526 observations is shown in table 2 and is illustrated graphically in figure 1.

The observations quoted are sufficient to indicate that the temperature of sheep may vary between rather wide limits, though the bulk of them lie between very much narrower limits.

*Normal temperature of sheep.* While no attempt will be made to discuss the literature on this subject in detail, it may be well to quote a few of the statements made as to the normal temperature. These are given in table 1. Some of the temperatures are given by the authors in degrees Centigrade. For the sake of comparison these have been converted into degrees Fahrenheit.

The normal temperature varies within wide limits and is dependent on many factors. This is probably the reason for the varied statements of different authors. In the records here discussed the lowest temperature was 98° taken at 8:20 a.m., June 23, 1925, on sheep 892, a yearling ewe. This sheep frequently had a low temperature in the morning. The highest

temperatures recorded were 106.7° in the case of sheep 951 at 8:52 a.m., August 24, 1926, and 106.8°, taken on sheep 859, a wether lamb at 5:22 p.m., August 27, 1924, at a time when the lamb was in a warm sunny pen.

TABLE 1

*Normal temperature of sheep as given by different authors*

| AUTHOR | TEMPERATURE, DEGREES FAHRENHEIT |
|---|---|
| Law (5, p. 22) | 103° to 104° |
| Smith, F. (8, p. 339) | 101.3° to 105.8° (Probably the majority lie between 103.6° to 104.4°) |
| Smith, Robert M. (9, p. 696) | 102.2° to 104° |
| Department of Agriculture (3, p. 741) | 103.1° |
| Siedamgrotzky (7, p. 123–124) | 101.3° to 107.24° (Average 104.36) |
| Hobday (4, p. 309) | 103.4° to 104.4° |
| Berneburg (1, p. 12) | 101.9° to 103.5° (Taken from table) |

TABLE 2

*Frequency distribution table showing temperature classes with the number of observations falling in each class of 8526 observations on the rectal temperature of normal sheep*

| TEMPERATURE CLASSES IN DEGREES FAHRENHEIT | | OBSERVED FREQUENCIES |
|---|---|---|
| Class limits | Mid-points | |
| 97.9– 98.4 | 98.15 | 3 |
| 98.5– 99 | 98.75 | 7 |
| 99.1– 99.6 | 99.35 | 33 |
| 99.7–100.2 | 99.95 | 119 |
| 100.3–100.8 | 100.55 | 379 |
| 100.9–101.4 | 101.15 | 1,074 |
| 101.5–102 | 101.75 | 1,817 |
| 102.1–102.6 | 102.35 | 1,988 |
| 102.7–103.2 | 102.95 | 1,702 |
| 103.3–103.8 | 103.55 | 904 |
| 103.9–104.4 | 104.15 | 344 |
| 104.5–105 | 104.75 | 100 |
| 105.1–105.6 | 105.35 | 28 |
| 105.7–106.2 | 105.95 | 17 |
| 106.3–106.8 | 106.55 | 11 |
| Total | | 8,526 |

In table 2 all of the observations have been arranged in the form of a frequency distribution table, as stated above. For each class the class limits, mid-points, and the number of observations are shown.

Figure 1 shows the distribution in the form of a frequency polygon.

From the frequency table and the accompanying histogram, it is at once apparent that, while occasionally a very low or very high temperature was recorded, as a rule, the observations fall within more restricted limits. For instance, of the 8,526 observations 5,507, or a little over 67 per cent fall between 101.5° and 103.2° Fahrenheit, while 7,485 or over 87 per cent lie between 100.9° and 103.8°.

The constants defining the center and the distribution of the total number of observations are as follows: These have been carried out to two places of decimals.

| | |
|---|---|
| Mean | $102.31 \pm 0.08$ |
| Median | $102.35 \pm 0.01$ |
| Distance from mean to mode | $0.06 \pm 0.01$ |
| Mode | 102.25 |
| Standard deviation | $1.00 \pm 0.005$ |
| Skewness | $0.06 \pm 0.01$ |
| Kurtosis | $0.27 \pm 0.04$ |

Inspection of the constants defining the center of the frequency polygon, the mean, median and mode shows the difference between them to be small, but still when their probable errors are considered, the difference between them would appear to be significant.

In fitting a curve to the observed data, the formula for the normal curve, $y = \frac{N}{\sigma\sqrt{2\pi}} \cdot \frac{1}{e^{x^2/2\sigma^2}}$, was used. The resulting curve as well as the distribution of the observations is shown in figure 1. In this the solid continuous line gives the computed curve, while the histogram represents the observations. The agreement between them is fairly close.

*Temperature and sex.* While the large majority of the sheep used were ewes, there were one or more wethers in most of the age classes. The means and standard deviations with their probable errors have been computed separately for the ewes and wethers of each age class, as well as for all ewes and all wethers of all ages. These, together with the numbers of animals and observations are given in table 3.

While the mean temperature for all the wethers is slightly higher than that for all the ewes and while this difference when considered in connection with the probable errors of the two means would perhaps appear significant this conclusion is not confirmed by a similar comparison of each of the age classes. Such a comparison is possible in seven age classes. While in four of these the mean temperature for wethers is slightly higher than that for ewes, when the probable errors are considered the difference in only one—the one year old class— is large enough to be of probable significance. On the other hand, in the five year old class the mean temperature for ewes is 0.47°F. higher than that for the single wether in this class. This is over

seven times the probable error of the mean temperature for the wether. In this case, however, observations were taken on only one wether, consequently little importance can be attached to it.

Taken as a whole, then there would appear to be very little difference between the mean temperatures for ewes and wethers, and the same thing holds good for the standard deviation.

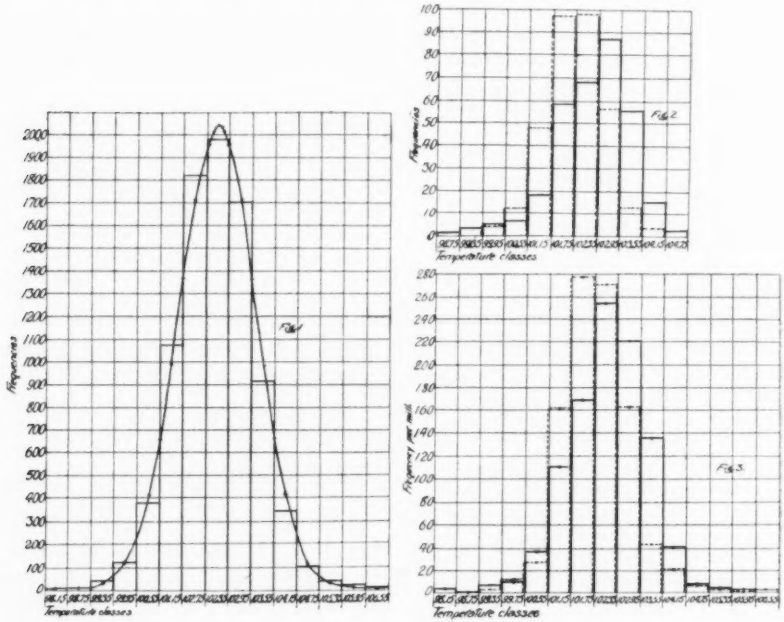


Fig. 1. Histogram showing frequency distribution of 8,526 observations on rectal temperature on normal sheep and the theoretical curve fitted to it.

Fig. 2. Histograms for two series of temperature observations on 41 sheep confined at two different periods of four days each. The solid line is for the observations during the first period. The broken line is for the observations during the second period.

Fig. 3. Histograms for two series of temperature observations on sheep kept in for eight days. The solid line is for the observations on 54 sheep kept in for the first time. The broken line is for the observations on 28 sheep kept in for the second time.

*Temperature as affected by age.* The observations were made on sheep of various ages. In order to study the effect of the change of temperature associated with age, they were divided into the age groups as shown in table 3. The age of sheep up to and including four years can be told by the teeth with sufficient accuracy for most purposes. After four years their

age can only be estimated. In the five year old group of table 3 are included those having all their permanent teeth but showing little wear. Those whose front teeth were worn down to the gums or had been lost are

TABLE 3

*Table showing the mean temperature and standard deviations for the ewes and wethers for the various age classes, for all ewes and all wethers together, and for all animals in each age class, with the number of sheep and observations for each group*

| CLASS | | NUMBER OF SHEEP | NUMBER OF OBSERVATIONS | MEAN | STANDARD DEVIATION |
|---|---|---|---|---|---|
| All ewes | | 435 | 7,244 | 102 28 ± 0 01 | 0 97 ± 0 01 |
| All wethers | | 70 | 1,154 | 102.47 ± 0 02 | 1.13 ± 0 02 |
| All sheep | | 514 | 8,526 | 102.31 ± 0 01 | 1.00 ± 0 01 |
| Lambs | Ewes | 26 | 490 | 103 00 ± 0 03 | 1.02 ± 0 02 |
| | Wethers | 20 | 410 | 102 92 ± 0 03 | 1 03 ± 0 02 |
| | Total | 46 | 900 | 102.96 ± 0 02 | 1.03 ± 0 02 |
| One year old | Ewes | 193 | 3,354 | 102.31 ± 0 01 | 0.96 ± 0 01 |
| | Wethers | 22 | 230 | 102 51 ± 0 03 | 0 77 ± 0 02 |
| | Total | 215 | 3,584 | 102.33 ± 0.01 | 0.95 ± 0 01 |
| Two years old | Ewes | 50 | 831 | 102.23 ± 0.02 | 0 99 ± 0.02 |
| | Wethers | 9 | 137 | 102 48 ± 0 07 | 1 21 ± 0 05 |
| | Total | 59 | 968 | 102.26 ± 0.02 | 1.03 ± 0 02 |
| Three years old | Ewes | 24 | 437 | 102.19 ± 0.03 | 0.80 ± 0 02 |
| | Wethers | 4 | 31 | 102 04 ± 0 10 | 0 84 ± 0 07 |
| | Total | 28 | 468 | 102.18 ± 0.03 | 0.80 ± 0.02 |
| Four years old | Ewes | 52 | 872 | 102.16 ± 0 02 | 0.96 ± 0.02 |
| | Wethers | 13 | 179 | 102 24 ± 0 05 | 1 07 ± 0 04 |
| | Total | 65 | 1,051 | 102.17 ± 0.02 | 0.98 ± 0.01 |
| Five years old | Ewes | 18 | 507 | 102 04 ± 0 03 | 0 87 ± 0 02 |
| | Wethers | 1 | 153 | 101 57 ± 0 06 | 1 15 ± 0 04 |
| | Total | 19 | 660 | 101.93 ± 0.03 | 0.96 ± 0.01 |
| Old | Ewes | 67 | 656 | 102.11 ± 0.02 | 0.88 ± 0 02 |
| | Wethers | 1 | 14 | 102 39 ± 0 14 | 0 78 ± 0 10 |
| | Total | 68 | 670 | 102.12 ± 0.02 | 0.88 ± 0.02 |
| Very old | Ewes | 5 | 97 | 101.81 ± 0.07 | 0 97 ± 0 05 |
| | Wethers | None | | | |

here classed as very old. Those between the five year old group and the "very old" were simply classed as old. Thus it is evident that there is a large chance for error in the "five year old" and the "old" groups. This

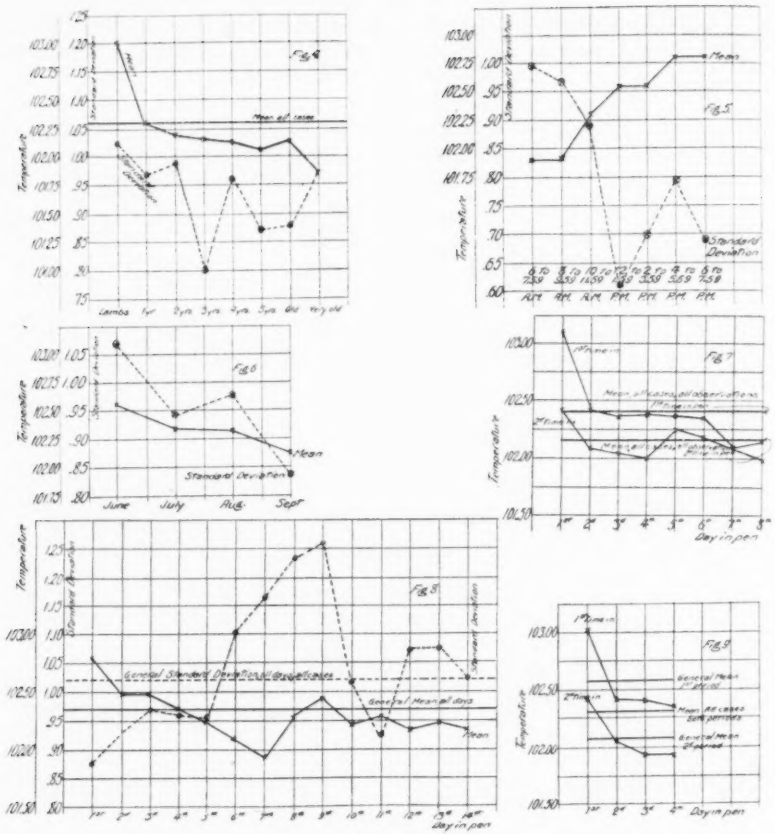

Fig. 4. Graphs showing the variations in means and standard deviations of temperature observations on sheep as influenced by age.

Fig. 5. Graphs showing the means and standard deviations of temperature observations taken at different times during the day.

Fig. 6. Graphs giving the means and standard deviations of temperature observations taken in different months during the summer.

Fig. 7. Graphs showing the changes in the daily mean temperature of sheep kept confined for 8 days the first time they had been under observation, as compared to the second period of confinement.

Fig. 8. Graph showing the changes in daily means and standard deviations of temperature observations on 26 sheep kept confined for 14 days.

Fig. 9. Graph showing the daily means of temperature observations on 41 sheep kept confined at two different periods of 4 days each.

chance is increased by the records being made in different years and to some extent by different observers. The rate at which sheep's teeth wear down, is probably dependent on more than one factor, so that no definite age can be assigned to the last two age groups.

Table 3 shows the number of ewes, wethers, and the total number of sheep, as well as the number of observations on each, for each age group, together with the mean temperature and standard deviation for each "sex-aged" class, and for all sheep in each age class.

For comparison there is also given the means and standard deviations for all observations on all ewes taken together, for all wethers taken together, and for all the sheep taken together without regard to age. In this last class it will be noted that the total number of sheep is greater than the total number of ewes plus the wethers, and similarly the total number of observations on this group is greater than the number of observations on all

TABLE 4

*Table showing grouping of temperature observations according to the time of day and giving the number of observations for each period together with the mean and standard deviation and their probable errors for the frequency distribution of each period*

| PERIOD | NUMBER OF OBSERVATIONS | MEANS | STANDARD DEVIATION |
|---|---|---|---|
| 6 to 7:59 a.m. | 151 | 101.84 ± 0.05 | 0.99 ± 0.04 |
| 8 to 9:59 a.m. | 4,080 | 101.87 ± 0.01 | 0.97 ± 0.01 |
| 10 to 11:59 a.m. | 277 | 102.29 ± 0.04 | 0.90 ± 0.03 |
| 12 to 1:59 p.m. | 27 | 102.55 ± 0.08 | 0.61 ± 0.06 |
| 2 to 3:59 p.m. | 74 | 102.57 ± 0.05 | 0.70 ± 0.04 |
| 4 to 5:59 p.m. | 3,608 | 102.79 ± 0.01 | 0.79 ± 0.01 |
| 6 to 8 p.m. | 181 | 102.79 ± 0.03 | 0.69 ± 0.02 |

The means and standard deviations are shown graphically in figure 5.

the ewes plus that on all the wethers. This is due to the fact that a number of observations on sheep whose age and sex records were incomplete, are included.

The means and standard deviations of the age groups of ewes are shown graphically in figure 4. Here the ewes have been taken in preference to all sheep because there are many more observations on them than on wethers, and because only ewes were included in the "very old" group.

The graph showing the mean temperature brings out clearly the gradual reductions in temperature associated with age. Apparently the greatest reduction occurs during the first year, but continues throughout the life of the sheep. The mean temperature of the "old" group is somewhat higher than would be expected and not quite on the line connecting the means of the other age groups. This is perhaps in part due to the errors in recording

the ages of the individuals in this age group. However, when the probable error is considered the mean temperature of this group is in fair agreement with the others. In general the temperature is highest in lambs, having a mean in our cases of 103.00°F., is reduced quite rapidly during the first year and then is reduced more slowly until the sheep reaches advanced years.

The variability as measured by the standard deviation differs considerably in the different groups and perhaps nothing positive can be said about it. The general trend as shown in figure 4 seems to be downward; that is, young sheep appear to be somewhat more variable as regards body temperature than older animals.

*Temperature and time of day.* While in the general routine of taking temperature observations, the largest part of them were taken between 8 and 10 a.m. and between 4 and 6 p.m., a sufficient number were taken at other times of day to furnish a basis for comparison. Consequently the day was divided into two hour periods and all observations falling within each period arranged in the form of frequency distribution tables and their means and standard deviations determined. Table 4 gives the data.

TABLE 5

*Table showing mean temperature and standard deviations, together with their probable errors by months. The numbers of animals and observations are included*

| MONTH | NUMBER OF SHEEP | NUMBER OF OBSERVATIONS | MEAN TEMPERATURE | STANDARD DEVIATION |
|---|---|---|---|---|
| June | 140 | 1,820 | 102.51 ± 0.02 | 1.06 ± 0.01 |
| July | 293 | 2,470 | 102.37 ± 0.01 | 0.94 ± 0.01 |
| August | 264 | 2,584 | 102.27 ± 0.01 | 0.97 ± 0.01 |
| September | 247 | 1,520 | 102.03 ± 0.01 | 0.84 ± 0.01 |

It is at once apparent that there is an increase in temperature between early morning and late in the afternoon, amounting, on the average, to nearly a degree Fahrenheit. This is in accordance with former observations. In these cases the period of increase was between 8:00 a.m. and 6:00 p.m. At least there is little difference between the 6:00 to 7:59 a.m. and the 8:00 to 9:59 p.m. observations, and still less between those taken at 4:00 to 5:59 p.m. and between 6:00 and 8:00 p.m. Judging from these observations the low body temperature would occur between 6:00 and 8:00 a.m. and the highest temperature between 6:00 and 8:00 p.m. In these cases the lowest average is 101.841 for the 6:00 to 7:59 a.m. group and the highest is 102.792 for the 6:00 to 8:00 p.m. group.

Apparently, too, there is a change during the day in the relative varia-

bility as shown by the standard deviation of the frequency distributions at the different periods. While the line (fig. 5) is far from a straight line it has a distinctly downward trend. The greatest variability was in the early morning group and the smallest in the afternoon periods. The smallest standard deviation was for the 12:00 to 1:59 p.m. group. This group, however, consisted of only 27 observations and when considered with the probable error is not significantly different from those for the groups taken later in the day.

Similarly the 2:00 to 3:59 p.m. group when the probable error is considered is not significantly different from the standard deviation of the 4:00 to 5:59 p.m. group. Taking this measure of variability into consideration for all the groups, it would appear that, so far as these records are concerned, there was less variability in the observations taken in the latter part of the day than those taken in the early part. In these cases, as the mean temperature increased the variability decreased. Where there is so little difference in the means for the different groups, of course, the coefficients of variation for the different groups will show the same general relative differences as is shown by the standard deviation.

*Temperature as affected by time of the year.* These observations were all made during the four summer months. In order to compare the average temperature during different periods of the summer, the observations made in each of the months of June, July, August and September have been treated separately.

In table 5 there is shown the means and standard deviations, together with their probable errors for all the observations taken in each of the four months. The number of animals and the total number of observations from which these constants were obtained are also given. The observations are for all the years. The number of observations made on a given animal varied between one and 60.

Figure 6 gives the change in the mean temperature and the standard deviations graphically.

In this table it is at once apparent that the highest mean temperature occurred in June and the lowest in September with the means for July and August having values nearly midway between them. Similarly the relative variability is smaller in September than in June. Taking the means for the different months at their face value one might conclude that sheep had a high body temperature in the spring which gradually decreased until late summer. Unfortunately other factors influence these figures and perhaps they give an entirely wrong impression.

Some of these influences are discussed on the following pages. At this point it should be pointed out that all or nearly all of the June observations were on animals that had never been handled in this manner before and were in the pens for the first time, while 91 of the 293 animals in the July

group, 245 of the 264 in the August group, and all but 15 in the September group had been in the pens and under observation during one or more previous periods. With these facts in mind it is probably safe to conclude that there is little if any difference in body temperature during the four months under consideration.

*Temperature as affected by confinement.* It has been the general opinion among those making the observations on the sheep that a gradual lowering of body temperature occurs for a few days after the animals are brought in from the pasture and kept in the pens. This has been quite noticeable in some cases. Partly because of this idea more care has been taken to obtain observations for some days after the animals were kept in and before they were used for an experimental feeding.

In looking over the notes for information bearing on this question, records were found of 26 sheep which were kept for 14 days or more in the pens before being fed plants which were, or might be, injurious, and on which both morning and afternoon temperature observations were made each day.

These sheep were of various ages and the observations were made at various times in June, July, August and the early part of September. While 17 of the animals were in the pens for the first time, the others had been in before, and were therefore more or less accustomed to being handled. It is also the fact that several of the sheep were more or less frightened or nervous, especially when the first few observations were taken.

The temperature observations on these animals for each of the first fourteen days were arranged in the form of frequency distribution tables. By combining the morning and afternoon observations for each day, frequency distributions for each day of confinement were obtained, and by combining all the observations on all the animals for the 14 days a frequency distribution was obtained showing the manner in which these observations, 728 in all, were distributed. These observations then gave a series of frequency distributions for which the means and standard deviations were computed.

Table 6 gives the mean and standard deviations for each day, and for all the observations together with their probable errors. Under the heading "Day of confinement" is shown the relationship of the day to the time when the animals were kept in. For instance "1st day" is the first day the sheep were in the pen "2nd day" the second day they were in, etc.

The important point to be noted here is the lowering in the body temperature at first and its continuing from the 10th day on at a fairly constant level at very close to 102.25° Fahrenheit.

This should be considered in connection with the mean of all the temperature observations made on all the sheep in the various years. This it will be remembered is 102.31. As the general mean for all the observations on

this group of sheep is very close to the mean for all the observations on all the sheep previously considered, it would appear that the 26 head offered a fair sample of the sheep in general. If this is true, it would appear that 102.2°F. would be very close to the mean temperature of sheep in confinement, accustomed to being handled and to the type of feed given these animals.

The means of the 52 observations for each day of confinement, and the standard deviations are shown graphically in figure 8. In this the somewhat irregular continuous line connects the means for the different days, while the dotted line connects the standard deviations. The horizontal solid line represents the general mean for all the observations taken on all

TABLE 6

*Table giving mean temperature and standard deviation for two observations daily on 26 sheep which were kept in pens for 14 days*

| DAY OF CONFINEMENT | MEAN TEMPERATURE | STANDARD DEVIATION |
|---|---|---|
| 1st day | 102.79 ± 0.08 | 0.89 ± 0.06 |
| 2d day | 102.49 ± 0.09 | 0.93 ± 0.06 |
| 3d day | 102.49 ± 0.09 | 0.98 ± 0.06 |
| 4th day | 102.36 ± 0.09 | 0.97 ± 0.06 |
| 5th day | 102.26 ± 0.09 | 0.96 ± 0.06 |
| 6th day | 102.18 ± 0.10 | 1.10 ± 0.07 |
| 7th day | 102.10 ± 0.11 | 1.16 ± 0.08 |
| 8th day | 102.29 ± 0.12 | 1.23 ± 0.08 |
| 9th day | 102.41 ± 0.12 | 1.26 ± 0.08 |
| 10th day | 102.23 ± 0.10 | 1.02 ± 0.07 |
| 11th day | 102.30 ± 0.07 | 0.93 ± 0.06 |
| 12th day | 102.19 ± 0.10 | 1.07 ± 0.07 |
| 13th day | 102.23 ± 0.10 | 1.07 ± 0.07 |
| 14th day | 102.19 ± 0.10 | 1.03 ± 0.07 |
| For all days | 102.32 ± 0.06 | 1.03 ± 0.02 |

the sheep during this period, while the horizontal broken line represents the standard deviation for the same set of observations.

The line connecting the means for the different days shows a distinctly downward tendency for the first seven days when it goes up somewhat and then remains fairly constant at very close to the 102.25 line, or slightly below the mean for all the observations considered in this curve. It is also of interest to note that this is slightly below the mean for all the observations made on normal sheep during the twelve years. It probably represents more nearly the normal temperature of the sheep which were confined in pens at the station where these observations were made, and fed and handled as these sheep were handled, than does the mean for all the observations.

Comparatively few of the 8,526 observations, which were used in computing the general mean temperature for all the sheep, were from animals which had been in the pens ten days or more. The larger proportion were made within the first week of confinement, consequently the mean of all cases would be greatly influenced by the observations made within the first few days of confinement and would be correspondingly high.

As seen in the graph, figure 8, the relative variability as measured by the standard deviation was itself somewhat variable, but, taken as a whole, it appears to have had a slight tendency to increase with the period of confinement. When the probable errors are considered the difference in the values of this measure of variability would appear to be of doubtful significance.

*Effects of confinement modified by other factors.* In considering the change in mean temperature for the first few days of confinement several factors should be considered, and an effort made to measure them. The obvious factors are, change of feed, confinement, and excitement or fright, and perhaps the amount of feed eaten.

These sheep before being placed under observation were kept during the day time in a pasture where there was an abundance of green feed and a varied diet. When under observation they were placed in pens ten feet square, and fed what alfalfa hay they would eat, and sometimes given a little bran. Once each day they were allowed to run in a small corral for an hour or two, usually between two and four p.m. where they had access to water and salt. The three lambs were an exception to this for they were allowed to run in and out of the pen at will and had access to green feed. Their diet was but little changed.

One half of these animals showed evidences of excitement, nervousness or fright when the first few observations were being made. This might be expected in range sheep which were unused to being handled and were in strange surroundings.

In the experimental work many of the sheep were used more than once for experimental purposes. After an animal had recovered from the effects of an experiment, or when it was evident no injurious effect was produced, the animal was returned to the pasture for a period. This period, as a rule, amounted to about a month. When returned to the pen a new series of observations was often taken to obtain a record of the temperature, pulse, and respiration to compare with the same bodily functions after the plant, in question, had been fed. In this way records for several sheep at two or more periods were obtained. Records were found of 41 adult sheep on which there were two separate series of observations for four or more days. These sheep were of all ages there being, however, more yearlings than those of any other age class. The observations used in the "first time in" series were taken in June, July and a few in the first part of August.

Those for the "second time in" series were very largely made in August and September.

If the higher temperatures found on the first days of confinement as shown in the preceding curve for the 14 days are due entirely to fright or excitement, because of being unused to the surroundings, or of being handled, then the first series of observations should show a greater difference between the mean temperature the first and succeeding days than should be obtained in the second series.

If, on the other hand, the falling off in temperature is due to the change in feed or to being kept in very restricted quarters, the changes should be approximately the same in the two series. The thing to do then is to compute the mean temperatures for the different days of confinement for the two series.

TABLE 7

*Table showing mean temperature and standard deviation of all observations in both series, of sheep kept in two periods of four days each, also the same constants of each 4 day series, and of the observations for each day of confinement in each group*

| GROUPING OF OBSERVATIONS | MEAN | STANDARD DEVIATION |
|---|---|---|
| All observations in both series (656 in all) | 102.32 ± 0.02 | 0.90 ± 0.02 |
| All observations in first series. Animals in for the first time | 102.56 ± 0.03 | 0.93 ± 0.02 |
| All observations in second series. Animals in the second time | 102.08 ± 0.03 | 0.73 ± 0.02 |
| First series, first day | 103.00 ± 0.06 | 0.85 ± 0.04 |
| First series, second day | 102.43 ± 0.06 | 0.81 ± 0.04 |
| First series, third day | 102.45 ± 0.07 | 0.96 ± 0.05 |
| First series, fourth day | 102.36 ± 0.07 | 0.93 ± 0.05 |
| Second series, first day | 102.43 ± 0.01 | 0.09 ± 0.005 |
| Second series, second day | 102.04 ± 0.05 | 0.68 ± 0.04 |
| Second series, third day | 101.97 ± 0.05 | 0.73 ± 0.04 |
| Second series, fourth day | 101.97 ± 0.06 | 0.82 ± 0.04 |

The means and standard deviations for the different days of confinement in the two groups and for each group are given in table 7.

In the figure 9 the changes in the mean temperature during confinement in the two periods are shown graphically. In this it is at once seen that the mean temperature for the second period was almost exactly one-half degree below that of the first period, that this difference holds good for each day, and that in both periods there was a gradual reduction in temperature amounting to 0.64° in the first period and 0.46° in the second. In other words, the decline was slightly greater in the first than in the second period.

In comparing the standard deviations for the two periods, it is quite

apparent that the temperature during the first period was much more variable than during the second period, and the difference, when considered with the probable errors is apparently significant. This difference again holds good for the two groups, as a whole, and for each day of confinement considered by itself. These results are surprisingly uniform.

The distribution of these observations about the means in the two groups is of interest because of the differences in the two groups. All of the observations for each period are shown in figure 2 in the form of histograms. This gives a chance to compare the distribution in the two groups more easily. The frequency polygon shown in solid lines is that for the first period or the 328 observations during the first period of four days. The polygon shown in dotted lines is for the second period of confinement when the sheep were more accustomed to being handled.

The polygon for the second period is of the normal symmetrical type. That for the first period is distinctly asymmetrical, the mean being more towards the upper end. In other words, the curve for this distribution is a skew curve in the negative direction. The positions of the two histograms on the X axis is somewhat different, that for the "second time in" series being shifted somewhat towards the left, or lower temperature values, as compared with the histogram for the "first time in" series.

As a further test of the differences in body temperature between sheep kept in the pens for the two periods, calculations were made for other sets of cases. In the notes, records of 54 sheep were found which were held eight days or more for the first period in the pens, and on which both morning and afternoon observations were taken each day. Similarly there were 28 animals which were in the pens for the second time and on which similar observations were made.

These sheep varied in age much as those used in obtaining the constants shown in table 7. Of the 28 sheep included in the group which were in the pen for the second time, 14 were individuals which were used in the group of 54 sheep which were in the pens for the first time. These 14 sheep then were in the pens at two different periods of at least 8 days each.

In 11 cases the same observations from the same sheep were used in the eight day "1st time in" confinement series as had been used in the 14 day series and the observations on two of the sheep used in the eight day "2nd time in" series, had been used in the 14 day test.

The means and standard deviations, together with their probable error, for each of these two groups and for each day of confinement were computed. The results are shown in table 8.

Here as in the case of the 41 sheep kept in pens for four days at each of two separate periods, there is a distinct difference in the mean temperatures, that for the "second time in" group being the lower. Taking all the observations in each group, the mean for the "second time in" is lower

than that for the "first time in" by a sum equal to ten times the probable error. Comparing each day of confinement in the two groups, it is seen that the means are somewhat lower for each day in the "second time in" series. This is brought out in the graph, figure 7. In this, it is apparent that the difference between the temperature means of the two groups is greatest the first day of confinement and gradually becomes less until the 7th day when the two means are approximately the same. In other words, where a number of sheep are kept confined in pens, on dry feed and under

TABLE 8

*Table showing means and standard deviations for the two sets of observations on sheep kept in for eight day periods*

The sheep used in the two periods are not necessarily the same sheep. Fifty-four animals were used in the "first time in" series and 28 animals in the "second time in" series.

| GROUPING OF OBSERVATIONS | NUMBER OF OBSERVATIONS | MEAN | STANDARD DEVIATION |
|---|---|---|---|
| All observations, 1st time in | 864 | 102.40 ± 0.02 | 0.97 ± 0.02 |
| All observations, 2nd time in | 448 | 102.13 ± 0.03 | 0.86 ± 0.02 |
| 1st time in 1st day | 108 | 103.09 ± 0.06 | 0.85 ± 0.04 |
| 1st time in 2d day | 108 | 102.41 ± 0.05 | 0.82 ± 0.04 |
| 1st time in 3d day | 108 | 102.37 ± 0.05 | 0.79 ± 0.04 |
| 1st time in 4th day | 108 | 102.39 ± 0.05 | 0.83 ± 0.04 |
| 1st time in 5th day | 108 | 102.36 ± 0.06 | 0.91 ± 0.04 |
| 1st time in 6th day | 108 | 102.34 ± 0.07 | 1.05 ± 0.05 |
| 1st time in 7th day | 108 | 102.09 ± 0.07 | 1.06 ± 0.05 |
| 1st time in 8th day | 108 | 102.15 ± 0.07 | 1.07 ± 0.05 |
| 2d time in 1st day | 56 | 102.41 ± 0.04 | 0.46 ± 0.03 |
| 2d time in 2d day | 56 | 102.08 ± 0.06 | 0.67 ± 0.04 |
| 2d time in 3d day | 56 | 102.05 ± 0.07 | 0.73 ± 0.05 |
| 2d time in 4th day | 56 | 102.01 ± 0.07 | 0.77 ± 0.05 |
| 2d time in 5th day | 56 | 102.25 ± 0.10 | 1.13 ± 0.07 |
| 2d time in 6th day | 56 | 102.18 ± 0.08 | 0.87 ± 0.06 |
| 2d time in 7th day | 56 | 102.07 ± 0.09 | 1.01 ± 0.06 |
| 2d time in 8th day | 56 | 101.95 ± 0.08 | 0.93 ± 0.06 |

observation for a period of time, the mean or average body temperature tends to become somewhat lower and to approach a given value. This for the two groups of adult sheep of various ages used in these tests was approximately 102.1° Fahrenheit.

Apparently the factor of fright, nervousness or newness of surroundings is an important one in determining the body temperature. This is more or less definitely measured by the difference between the means for the first day of confinement in the two series. The fact that the sheep were somewhat accustomed to being handled and to being in pens the second time

they were brought in probably accounts for the differences in the means of the temperature observations for the first day in the two series. This difference in both the 4 day and the 8 day series is very similar. In the 4 day series, it was 0.57° Fahrenheit and in the 8 day series 0.68° Fahrenheit.

Apparently this factor, on the average, influences the temperature for about a week where the animals are in the pens for the first time.

The somewhat slight reduction in temperature during the first day or two of the second period of confinement may have been in part due to the change of feed and chances for activity. This is not, however, very apparent.

In these cases as in those of the four day tests, the relative variability as shown by the standard deviations is somewhat greater for the "first time in" series than for the observations taken in during the second period. The difference to be sure is not great, being only 0.11°F. This would appear significant, however, when the probable errors are considered. In general, too, these differences hold for the different days, and agree with the results in, the four day series previously discussed.

For the sake of more convenient comparison the relative frequencies per thousand are shown graphically in the histograms, figure 3. In this it is seen that the two frequency distributions are of essentially the same character, but, in general, that for the "first time in" series has a slightly greater value on the abscissa than that for the "second time in" series, that is, the histograms is as a whole slightly farther to the right as ordinarily plotted. This difference in the position of the two histograms to be compared is practically the same as that for the four day test as shown in figure 2.

*Temperature and nervousness or excitement.* In a number of instances the person taking the observations added to the record that the animal was, nervous, excited, or frightened. As these records were made by various observers and as a variety of expressions were used, it is not safe to distinguish between the degrees of nervousness. All one can say is that the animal showed some reaction which the observer interpreted as indicating that the sheep was nervous, excited or frightened at the time the observation was being made. These records vary from such as "slightly nervous" or "uneasy" to "very much excited."

There were found 828 such records on a large number of sheep. These had a mean value of $102.83 \pm 0.02$ and a standard deviation of $96 \pm 0.02$. Of these, 315 observations on 193 different individuals were taken the first day of confinement and the first time the animals had been in the pens. These were found to have a mean value of $103.24 \pm 0.03$ and a standard deviation of $0.87 \pm 0.02$.

When these means are compared with the mean values of the other groups of observations they appear to indicate that excitement or fright

causes a rise in the body temperature. The mean for all the cases taken irrespective of degree of excitement or length of time of confinement is higher than that for any of the groups, except those for the "first day" first time in groups. In these two groups many of the observations were taken when the animals were more or less excited. The group of excitement cases taken the first day of confinement and the first time in the pens has a mean 0.24°F. higher than that for the corresponding day of the 41 sheep kept under observation for four days and 0.16°F. higher than that for the 54 sheep kept in 8 days. There is hardly sufficient difference between the standard deviations to be significant.

When one considers that it must take an appreciable amount of time for changes in body temperatures to take place and that usually an observer was in the pen with the sheep only three or four minutes while taking the observations, the differences due to excitement are fully as great as one would expect. From them it seems evident that nervous excitement or fright has a tendency to cause a rise in the body temperature.

## CONCLUSIONS AND GENERAL STATEMENT

1. A series of body temperature records on sheep may be arranged in the form of frequency distribution tables which when plotted take the general form of normal or Gaussian curve. Such a curve for the observations made at the Salina Experiment Station between the years 1915 and 1926 inclusive had a mean value of 102.31° Fahrenheit, a standard deviation of 1.00, is slightly skew in the negative direction and slightly more peaked than the normal curve.

While individual observations may be as low as 98°F. or somewhat higher than 106°F. a very large proportion of them fall between 100.9°F. and 103.8°F. and about 64 per cent between 101.5°F. and 103.2°F.

2. There is no appreciable and constant difference in body temperature between ewes and wethers.

3. The body temperature is highest in lambs and gradually decreases with age, the rate of decrease being greatest during the first year. The variability as measured by the standard deviation also decreases slightly with age.

4. Body temperature is lowest in the early morning and increases during the day time being on the average about 0.95°F. higher between 6 to 8 p.m. than between 6 to 8 a.m. During this same period the variability decreases.

5. When other factors are eliminated the mean temperature is about the same for the four summer months.

6. When confined in pens and observations are taken daily for several days the mean temperature falls for a period and then remains approximately the same, the rate of decrease depending on whether the animals are under observation for the first time or not. The decrease is greater

and continues longer the first time they are subjected to a series of observations than during subsequent periods.

When under observations for the first time it takes a week or more for the temperature to reach a fairly constant level. On the average this level is very close to 102.25°F. which may be taken as the normal average rectal temperature of sheep under the conditions existing in these cases.

At least two factors play a part: *1*, nervousness or fear causes an increase in body temperature, while 2, change from green feed and freedom to dry feed and confinement brings about a reduction in body temperature.

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# REACTIONS OF THE CROP (ESOPHAGEAL) MUSCLES UNDER TENSION, WITH A CONSIDERATION OF THE ANATOMICAL ARRANGEMENT, INNERVATION AND OTHER FACTORS

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All previous work on the crop or esophagus has disregarded the activity of the longitudinal muscle and the correlated responses of the circular and longitudinal muscles. These would seem to be important in the motor activity of this organ just as is the case with the same muscles in other portions of the alimentary tract. In fact, under the conditions of our experiments, the longitudinal muscle has generally shown a greater spontaneous activity and responsiveness to nerve stimulation and to drugs than the circular muscle. It is the object of this paper to discuss the arrangement, innervation and general physiological reactions of the pigeon crop muscles which we have used considerably in studies of smooth muscle responses in anaphylactic shock (1) and to drugs. It will be followed by other papers dealing more specifically with reactions of the muscles to drugs and with altered reactions brought about by changes in functional state.

ANATOMICAL ARRANGEMENT. Our work with the intact esophagus has been limited to the crop portion because it is so easily accessible for records of muscular movements and because it probably would be most commonly chosen in future work. The general anatomical features are sufficiently described in textbooks on comparative and fowl anatomy (2) which, however, do not give desired details about the innervation. We have, therefore, made extensive dissections and also some histological studies of the organ in pigeons. The main features may be summarized as follows: Our histological sections corroborate the detailed and extensive studies of the human esophagus by Greving (3). The structure of the crop is similar to other portions of the alimentary tract. There are the inner circular and outer longitudinal layers of smooth muscles and between these are extensive ganglionated nerve plexuses which probably extend into the submucosa and mucosa. The mucosal distribution is of interest because

[1] Submitted by E. M. Butt in partial fulfillment of the thesis requirement for the degree of Doctor of Medicine.

of the physiological responses to be described later on. It is interesting to note that there are considerable differences in the musculature of the esophagus in different species. According to Oppel (4), the esophagus in the rabbit and dog is composed almost entirely of striated muscle; in the cat, two-thirds of the upper part is striated muscle, and in man, one-third of the upper part. In apes, smooth muscle is found only in the lower part (Schilf, 4a). From the facts that the musculature of bird and human esophagus is very similar, and next to these is that of the cat, the physiological and pharmacological responses might be expected to be similar, this being further suggested from the brief report on cat esophagus by Prakken (12).

The vagus or parasympathetic innervation is well known to be similar to that of mammals and need not be described here, but the sympathetic innervation is scarcely discussed in text-books on comparative and fowl anatomy. Even in human anatomies, the sympathetic nerve supply to

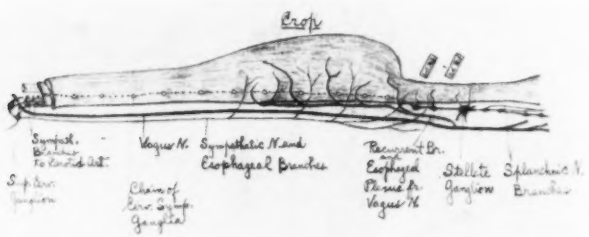


Fig. 1. Diagram of the autonomic nerve supply to the right side of the esophagus in pigeons.

the esophagus is not clearly detailed, but the recent careful studies of Greving (3) leave no doubt of a very extensive though complicated and variable innervation the full length of the organ. Our dissections of the pigeon crop and results of nerve stimulations indicate that there is a sympathetic nerve supply and function. Figure 1 illustrates diagrammatically the course, distribution and relations of the autonomic nerves on the right side of the esophagus. A detailed description is omitted in order to save space. It will suffice to draw attention to the presence of twelve cervical sympathetic ganglia in birds instead of the usual three in mammals, and to the two sources of the sympathetic nerve supply, i.e., from the cervical sympathetic and the thoracic ganglia. The cervical sympathetic nerve, which supplies the upper and crop portions of the esophagus, has its origin in the superior cervical sympathetic ganglion, and incidentally, sends no sympathetic supply to the iris in birds (confirmation of Zeglinski, 5). In the lower or thoracic portion of the esophagus, the sympathetic

innervation is derived from the stellate (first thoracic) ganglion and from two smaller ganglia lying just above the stellate. All three ganglia send many fine nerve filaments which form an intricate plexus.

Electrical stimulation of the vagus and sympathetic nerve trunks indicates that both nerves have controlling but different influences on the muscles. However, before discussing the functions of the nerves, the method of recording the muscular responses, the spontaneous activity of the muscles and their responses to pressure will be described.

METHOD OF RECORDING THE MUSCULAR RESPONSES. The arrangement used records the contractions of the circular and longitudinal muscles together. The details of the method and a diagram of the arrangement have been given in a previous paper by Hanzlik, Butt and Stockton (1). Briefly, the activity of the circular muscle is recorded from the stilus of a manometer containing carbon tetrachloride and joined by a stiff catheter to a balloon in the crop. The activity of the longitudinal muscle is recorded from a light heart lever which is joined by a fine thread passing over a pulley to a small figure "8" wire clamp attached to the crop in the region of the balloon, thus recording the movements in the horizontal plane. The points of the lever and the stilus are aligned so as to record the reactions of the longitudinal and circular muscles simultaneously.

The wire clip attached to the crop for longitudinal contractions requires a small skin incision which is made under light ether anesthesia, merely a whiff of ether sufficing. Since the introduction of the balloon requires no operating, it is obvious that the entire system is used under conditions of minimum trauma and anesthesia. The pigeon is tied to a small board, the head being held down by a simple device previously described (1), and usually remains quiet, but perfect quietude is obtained by curarization (1 to $1\frac{1}{2}$ cc. of 0.5 per cent curare extract), making a tracheotomy and using artificial respiration at the same time.

With the balloon in position, but without distention, the system records no movements from the muscles. However, on inspection the longitudinal muscle, just above the crop, is frequently seen to execute local, short and rather rapid but ineffective contractions and relaxations. These do not seem to sweep beyond the crop which contains the balloon. The pressure of the catheter may promote these local contractions, but since they do not seem to involve the entire esophagus and have been seen also in the totally empty crop it is not believed that the catheter plays an important rôle in their causation. The circular muscle appears to be quiet, the esophagus appearing contracted, or at least small in caliber. However, the moment the crop is distended, the levers may record spontaneous activity and the crop muscles become more sensitive to stimuli. The remainder of the paper will deal with the first, or reciprocal, response of the muscles to pressure and various factors affecting the activity of the

muscles without, however, paying special attention to peristalsis of the organ as a whole, such as spreading of the wave of contraction, propulsion, etc.

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Fig. 2. Successive relaxations of longitudinal muscle with increasing distention of the living crop (*A*) contrasted with a nearly complete lack of response to the same pressures in the same crop, twenty-four hours post mortem (*B*).

Shows that the relaxation of longitudinal muscle is functional and not mechanical. The pigeon was curarized and vagus nerves cut.

In all figures, tracings read from left to right; time, each stroke = 5 seconds; *Circ.* = circular muscle, and *Long.* = longitudinal muscle.

Fig. 3. Abolition of reciprocal relaxation of longitudinal muscle after cocainization of crop mucosa (expt. 157).

Shows that the relaxation is due to a local myenteric reflex. The pigeon had been curarized and autonomic nerves cut from the start. *A*. Control relaxations of longitudinal muscle from application of increasing pressures ($CCl_4$) in the crop. *B*. First part, tremors from absorbed cocaine and additional curare intravenously; second part, test of longitudinal muscle responses to pressures in the crop 6 minutes after cocainization of crop mucosa: relaxations practically absent.

Fig. 4. Spontaneous peristaltic contractions of the circular and longitudinal muscles of the pigeon crop under different pressures of carbon tetrachloride ($CCl_4$), (expt. 59).

Shows coördinated, simultaneous, reciprocal relaxations of the longitudinal muscle with each contraction of the circular muscle. The pigeon was curarized and the vagus nerves cut.

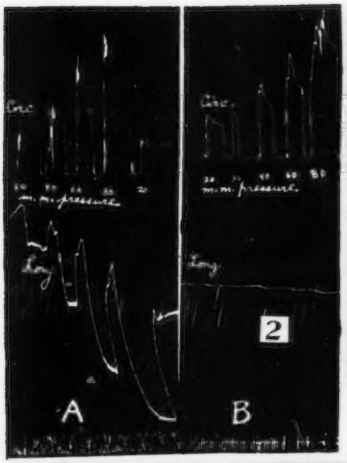
Fig. 5. Types of crop muscle responses to direct vagus nerve stimulation. *A*. Reciprocal response of the muscles during faradization of the right vagus nerve (*v.s.*), i.e., contraction of the circular with simultaneous, marked relaxation of the longitudinal muscle (expt. 150). *B*. Fleeting, small reciprocal response of the muscles followed by a marked contraction of the longitudinal muscle with peristaltic contractions of both muscles (expt. 151).

Fig. 6. Contraction of both the circular and longitudinal muscles on electrical stimulation of the sympathetic nerves (*s.s.*) contrasted with a reciprocal response of the muscles on electrical stimulation of the vagus nerves (*v.s.*) in the same crop (expt. 98).

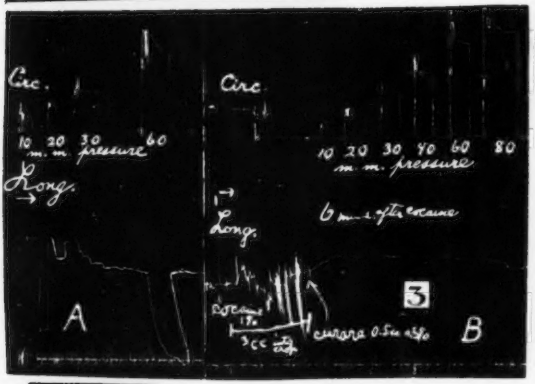
Qualitatively, the muscular responses in this experiment were typical, but quantitatively the response of the longitudinal muscle during sympathetic nerve stimulation was somewhat more marked than usual.

Fig. 7. Crop muscle responses to electrical stimulation of the thoracic cord under different conditions.

Shows that sympathetic innervation of the crop has augmentor influence only and that the influence of certain drugs on it is typical. *A*. Control: both muscles contract (expt. 189). *B*. Medulla pithed and vagi cut: both muscles contract, impulses being mediated through sympathetic nerves (expt. 188). *C*. Same crop after paralysis of vagi by atropine (intravenously): both muscles still contract showing that the sympathetic innervation was not affected by atropine and that the muscular response was not vagal; *v.s.* = stimulation of the peripheral vagus trunks. *D*. Typical effect of nicotine intravenously and absence of crop muscle response to thoracic cord stimulation after complete nicotinization, showing that the impulses from the cord were relayed through ganglia thus proving conclusively that the muscular responses were invoked through sympathetic nerve stimulation (expt. 190). *c* = thoracic cord stimulation.

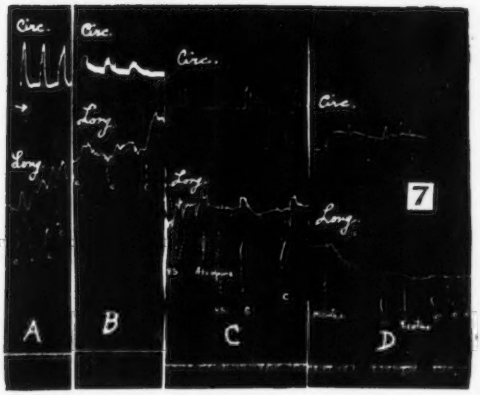

Circ.
m.m. pressure
Long.
A
B
2

Circ.
Long.
Pressure: 10 — C.C.
Pressure: 30 — C.C.
Spontaneous Peristalsis
4


Circ.
10 20 30 60 m.m. pressure
Long.
10 20 30 40 60 80 m.m. pressure
6 m.c. opticaine
3 cc
cocaine 0.5% 1%
A
B
3

Circ.
Long.
A
B
5

Circ.
Long.
6


Circ.
Long.
A
B
C
D
7

THE RELAXATION, OR RECIPROCAL RESPONSE, OF THE LONGITUDINAL MUSCLE ON DISTENTION OF THE CROP. The immediate response of the longitudinal muscle to distention of the crop is a relaxation which occurs in varying degree but is more or less proportional to the pressure. Following the relaxation, the longitudinal muscle contracts and generally continues with peristaltic movements. Meanwhile the circular muscle, which is under stretch and the recording stilus raised, remains quiet as a rule. The movements of the two muscles will be discussed in the next section.

As to the cause of the longitudinal relaxation, occurring in response to distention and also on sudden contraction of the circular muscle spontaneously, it is mainly functional and not mechanical, for the same arrangement of the muscles in the dead crop gives far too small a relaxation of the longitudinal muscle to different pressures applied in precisely the same way to the living crop. The degree of relaxation of the longitudinal muscle in a dead crop is illustrated in figure 2B. This should be compared with the typical relaxations of a living crop in A of the same figure. The difference between the two records is quite obvious. The presence of a small, though relatively insignificant, mechanical factor in the relaxation can not be denied, but this merely emphasizes that the main factor in the living crop is a functional one.

That the seat of the longitudinal relaxation is peripheral and not dependent upon the autonomic nerves was indicated by the facts that it still occurred in crops with these nerves cut and also after complete atropinization. That it is caused by a local nerve reflex was further shown by the fact that the main relaxation, except for the small mechanical factor, was abolished by cocainization of the mucosa in crops with nerves cut. The records in figure 3 illustrate the complete absence of the functional relaxation in a cocainized crop with nerves cut. It may also be compared with the record of the intact and untreated crop in figure 2A.

In the experiments designed to test the local reflex mechanism, relaxations of the longitudinal muscle towards progressively increasing distention pressures, as controls, were first recorded. The nerves in some pigeons were cut before the controls were secured and in others after the controls. Then 3 to 4 cc. of cocaine in 1 per cent strength were administered into the crop through a catheter and the crop balloon was allowed to collapse and swished around so as to spread the cocaine over the mucosa. In some crops, additional cocaine was injected into the crop at different places. Some pigeons responded in about five minutes with slight tremors, which were due to the cocaine, indicating that the cocaine had been absorbed and thus acted both locally and systemically. Other pigeons which were more completely curarized did not have tremors. At the end of about ten minutes, the same distention pressures were re-applied to the cocainized crop, but with no more relaxation of the longitudinal muscle than

could be ascribed to the small mechanical factor, as illustrated in figure 2B. It seems obvious that cocaine prevented the typical relaxation by paralyzing a nerve mechanism, for the muscles were not paralyzed as indicated by their responses later to drugs and electrical stimulation. Therefore, the cocaine eliminated a local nerve reflex which must be responsible for the relaxation caused by distention. The only nerve tissues capable of reflex responses are the myenteric plexuses which, of course, are present in the crop. These plexuses respond to the mechanical stimulus of distention just as they may respond to other kinds of stimuli. It is not unreasonable to assume from these results that the reciprocal response of the crop muscles, i.e., when the circular contracts and the longitudinal relaxes spontaneously, or under nerve stimulation, drugs, etc., is due to the invocation of the local myenteric reflexes. Thus, it appears that the myenteric nerve reflexes possess the function of coördinating the responses of the circular and longitudinal muscles to certain stimuli.

We have not attempted to remove the plexuses by mutilating operations in order to see if mechanical removal of them would accomplish the same result as did cocaine. Theoretically, this would seem to be the crucial test, but as a matter of fact the attempt to remove is no guarantee that removal is accomplished and the danger of obtaining abnormal and injured preparations is so great that the destructive method would not bring us nearer to the solution of the problem of the function of the myenteric plexuses. The difficulties attendant upon the removal of plexuses in excised intestines are evident enough from the attempts of Gunn and Underhill (6) and of Gasser (7). The L-shaped segments of excised intestines tried by Siaulis and Sollmann (8) would not seem to overcome important difficulties present in so-called "plexus-free" preparations, but these authors feel that, at least in their preparations, there is probably no neuromuscular coördination between the two limbs representing the circular and longitudinal muscles. This much can be said in favor of our crop preparations: they were virtually denervated (autonomic nerves), kept in good nutrition by a natural circulation and were not mutilated, and yet cocaine, which is predominantly a nerve poison, abolished the typical response to the pressure stimulus. This proof, therefore, is as good as any brought forward thus far to sustain the arguments for the functional importance of the myenteric plexuses first advanced by R. Magnus (9) from excised intestines. It would seem better than any negative evidence from abnormal preparations. In any case, our results with cocaine, if they are to be denied the importance we attach to them with respect to nerve plexuses, would require an unusual explanation of the action of this local anesthetic. Lacking the alternative of a suitable explanation for the results under our conditions we leave them with what seems to be the most obvious explanation. We may add, in anticipation of results with drugs in a later paper, that if the reciprocal responses of the longitudinal muscle to pressure and other kinds of stimuli were mechanical and our results with cocaine accidental, that the reciprocal action should occur invariably with all kinds of stimuli, but this is not the case. Since this is not the case, some stimuli break the reciprocal action or act beyond the plexuses, i.e., on muscle substance, as is the case with some direct muscular stimulants.

From the condition of distention pressure under which the relaxation of longitudinal muscle has been demonstrated it is easy to imagine what would happen in the esophagus, especially the crop portion, distended with food. Only a step further is

required to visualize the coördinating rôle of the myenteric plexuses in the muscular activity during movement and digestion of food. However, we have not concerned ourselves with the peristaltic function of the whole organ in which gradients of activity, propulsion, etc., would have to be considered. We may pass on to the spontaneous activity of the muscles under the more restricted conditions of our experiments.

SPONTANEOUS MUSCULAR CONTRACTIONS IN THE DISTENDED CROP. With the muscles under a distention pressure of 20 mm. of carbon tetrachloride, the pressure generally employed in our work, the muscles may or may not show spontaneous contractions together. In the presence of food, or in an unstarved pigeon, the muscles are apt to be quiet, but in starved pigeons there is usually some activity. For this reason it has been a routine practice to withdraw food from the pigeons for at least a day before using them. Of the two muscles it is the longitudinal which makes rather large and frequent but irregular contractions. These follow the initial relaxation on the application of distention. The circular contractions, as a rule, are slow and infrequent but generally more regular than the longitudinal. Frequently the longitudinal muscle contracts independently of the circular, which remains quiet and shows neither contractions nor tonus changes. We have practically never encountered a crop in which the circular muscle contracted independently of the longitudinal muscle under the conditions of our system.

It is possible that the greater activity of the longitudinal muscle recorded by our system is due partly to the smaller resistance against which the muscle works, since the movements are recorded by a light lever. On the other hand, the contractility is not appreciably different whether the muscle is under distention pressure or not, and the activity increases, if anything, with the pressure. This is not the equivalent of stretching of all the fibers equally at all points, but a stretch together with lateral pressure must occur in the region of the balloon from which the longitudinal record is made. In any case it is probable that our system records the optimum activity of the longitudinal muscle, and moreover the technique was the same throughout all our work.

When the two muscles have acted together spontaneously they have given records of coördinate, but opposite activity, that is, at the moment of circular contraction the longitudinal muscle has responded by a simultaneous relaxation. In other words, a reciprocal action occurred which is identical with the longitudinal relaxation in response to the distention stimulus described above. The contraction of the circular muscle however is not a distention, but it must act nevertheless as an adequate stimulus to the local reflex mechanism, arising from a pull of the contracting fibers on the plexuses. The degree of relaxation of the longitudinal muscle is variable but seemed to be more or less proportional to the degree of con-

traction of the circular muscle. Following each relaxation, the longitudinal muscle, as a rule, executes some contractions which may continue during the relaxation of the circular muscle, but these contractions are at once stopped and replaced by a sudden relaxation when the circular muscle begins to contract again. That the seat of the spontaneous peristalsis, and also of the reciprocal action, of the muscles is peripheral was plainly indicated by the fact that the phenomena still occurred after section of both the vagus and sympathetic nerves in the neck. A typical coördinated and reciprocal action of the two crop muscles occurring spontaneously, after nerve section, is illustrated by the tracings in figure 4.

When the usual peristalsis has begun, constriction bands may be seen moving from above downwards and terminating at the edge of the balloon. Between the peristaltic waves, which at this time may be regular in amplitude and rate, small, local, alternating contractions and relaxations may be seen. In crops, which exhibit no peristalsis, the esophagus, including the crop portion, manifests only tonus changes toward mechanical and drug stimuli. This condition of inactivity is present in recently fed pigeons and in crops containing food, but there are also active inhibitory reflexes which will stop the contractions of a crop during spontaneous peristalsis.

Reflexes. Rogers (10), using the balloon method with the pigeon's crop, claimed he could not record contractions unless the pigeons were blinded or placed into a darkened cage so as to eliminate the reflex inhibition of light. While we can corroborate the inhibitory efficiency of light when a beam is suddenly thrown into the eye of a pigeon, or by causing visual disturbances with moving objects, we have not found that it is necessary to cover the pigeon's head or place the animal in a box for demonstrating spontaneous peristaltic activity. We have repeatedly observed this type of activity in the ordinary daylight of the laboratory. Most of our pigeons have been curarized because muscular movements and reflexes, struggling and tremors, seemed to have a greater inhibitory influence than light. This could be shown in curarized pigeons by pinching a leg or toe, also by blowing cool air on the abdomen. Only occasionally did pinching a leg or toe, or stroking the feathers over the breast, cause a contraction of the circular muscle. The proof of the existence of central inhibitory impulses, which appeared to arise wholly centrally and were not evoked peripherally, was obtained by removing the cerebral cortex. Under these conditions, the spontaneous peristaltic activity of the circular and longitudinal muscles began more promptly and effectively, the vigor of the contractions being greater, than in pigeons with brain intact. Hypermotility and hypertonicity in the crop of starved decerebrated pigeons have been reported by Rogers (10). Decapitation, and destruction of the cord with crop nerves cut, also permitted spontaneous

peristalsis, but destruction of the cord stopped the effects of pinching and other reflexes from the trunk. The activity of the inhibitory reflexes may account for some of the irregularities in, and the difficulties of obtaining, spontaneous crop peristalsis.

RESPONSES TO AUTONOMIC NERVE AND SPINAL CORD STIMULATIONS. *Vagus nerves.* During the course of our work we have had the opportunity to test the vagus nerve function in 154 pigeons under different conditions. In 26 pigeons the vagi were severed in the upper cervical region and the peripheral and central ends prepared for electrical stimulation. The results were as follows: Stimulation of the central end produced no effect upon the crop. Stimulation of the peripheral end invariably resulted in a relaxation of the longitudinal and a simultaneous contraction of the circular muscles. In other words, there was a reciprocal response of the muscles analogous to that of distention of the crop. The relaxation of the longitudinal muscle was variable in degree. In some crops it was very marked though temporary, the recording lever returning to its previous level. In other crops, a short relaxation was usually followed by contractions. These results generally agree with those of Inouye (11) who found on stimulation of the vagus a contraction of the circular muscle and relaxation of the longitudinal. Occasionally a contraction of both muscles or a contraction only of the circular muscle with no change in the longitudinal muscle was observed. A close inspection of the curves showed that there would be a small downward nick in the longitudinal curve giving the impression that the relaxation phase was too small and evanescent for satisfactory record, thus recording chiefly the subsequent event of increased activity or only a cessation of activity. Comparisons of the reactive efficiency of the right and left vagi were made in 11 pigeons, using different strengths of faradic stimuli in the group, but the same strength for the nerves of each pigeon. The results are presented in table 1. Only the circular muscle was used in the majority of pigeons, since the longitudinal responses to vagus stimulation were more variable. It is seen that greater muscular responses were evoked in the majority, or 9, pigeons on stimulation of the left vagus nerve while in only 2 pigeons were greater responses manifested by stimulation of the right vagus. The effects of vagus stimulation on the crop muscles are illustrated in figure 5, and in figure 6, which contrasts the result with sympathetic nerve stimulation.

The effects of vagus stimulation on both muscles were abolished by injections of atropine, and also by nicotine and by large doses of papaverine, which paralyzed the muscles. They were greatly diminished by apocodeine, a drug which is thought to be more effective for abolishing sympathetic nerve functions than those of the vagi. The effects of these and other drugs will be more fully discussed in another paper, but, for the purpose of this paper, they indicate that the vagus nerves to the crop are affected by typical autonomic poisons, as in other regions and species.

*Sympathetic nerves.* Electrical stimulation of the right or left, or both, sympathetic nerves described in the fore-part of the paper under "anatomical arrangement" invariably resulted in a marked contraction of the longitudinal muscle, the circular muscle being usually unaffected and only occasionally showing a slight contraction. Figure 6 illustrates a result of sympathetic nerve stimulation. The slight contraction of the circular muscle may have been due to mechanical disturbance in the system caused by the marked contraction of the longitudinal muscle. The results presented in table 2 and obtained from 5 pigeons indicate the comparative responsiveness of circular and longitudinal muscles to electrical stimula-

TABLE 1

*Comparative responses of the crop muscles to equal stimulation of the right and left vagus nerves in the same pigeon**

| PIGEON | RIGHT VAGUS | LEFT VAGUS |
|---|---|---|
| 1 | C = +10; +12 | C = +15; +21 |
| 2 | C = +5 | C = +12 |
| 3 | C = +1 | C = +2 |
| 4 | C = +13 | C = +10 |
| 5 | C = +1 | C = +3 |
| 6 | C = +6 | C = +12 |
| 7 | C = +8 | C = +2 |
| 8 | C = +8; +5<br>L = +2; 0 | C = +6; +7; +9; +7<br>L = 0; 0; −6; −1 |
| 9 | C = +8<br>L = +4 | C = +12; +12<br>L = +4; +8 |
| 10 | C = +3<br>L = −6 | C = +3; +5<br>L = +5; 0 |
| 11 | C = +2<br>L = −5 | C = +3<br>L = +1 |

* The letters and signs in the table have the following meanings: C = circular muscle; L = longitudinal muscle; (+) = contraction; (−) = relaxation; the figures denote millimeters.

tion. It is seen that the height of longitudinal muscle contractions ranged from 14 to 71 mm. in response to the strongest faradic stimulus used, whereas the greatest contraction resulting from the same stimulus applied to the vagus nerves (table 1) was less than 10 mm. The figures for the circular muscle are strikingly negative, the greatest contraction recorded being 5 mm. In one trial, the right sympathetic nerve gave a greater contraction than the left nerve to the same strength stimulus. The sympathetic responses were abolished by large but not by small doses of atropine and nicotine, while very small doses of these drugs sufficed to paralyze the vagus nerves. They were not affected by ergotoxine.

These results indicate that the sympathetic nerves to the crop have an augmentory function, if anything, and this would seem to be limited to the longitudinal muscle. Both the functional result and the limitation of muscular innervation are of interest. It may be mentioned now, in anticipation of the results with drugs to be described in another paper, that epinephrin stimulates the untreated crop muscles, an effect in agreement with that of sympathetic nerve stimulation. The lack of an inhibitory response from sympathetic nerve stimulation to this (the crop) portion of the alimentary tract is not altogether surprising, because the nerves stimulated emerge partly from the superior cervical sympathetic ganglion whose functional influence is augmentory. However, in birds, there is no

TABLE 2

*Responses of crop muscles to sympathetic nerve stimulation**

| PIGEON | RIGHT SYMPATHETIC | LEFT SYMPATHETIC |
|---|---|---|
| 1 | | C = +2; +5; +2<br>L = +37; +44; +65 |
| 2 | C = +5; 0<br>L = +71; +67 | |
| 3 | C = 0; 0<br>L = +25; +26 | C = 0; 0<br>L = +14; +15 |
| 4 | | C = 0; 0<br>L = +65; +49 |
| 5 | C = +1<br>L = +40 | |

* The letters and signs in the table have meanings as follows: C = circular muscle; L = longitudinal muscle; (+) = contraction. The figures denote millimeters.

sympathetic supply to the iris and therefore our reasoning from this fact can not be given the proof which, of course, is easily obtained in all species having the nerve supply in the iris. That is, the sympathetic here constricts the radial muscles thus dilating the pupil. The nerves which we have stimulated in the neck are not the only sympathetic nerves to the esophagus, although, as far as the crop is concerned, the cervical nerves appear to be the only discrete sympathetic nerve trunks to the crop portion. From our dissections of pigeons, described in the forepart of the paper, there is also a sympathetic nerve supply from the stellate and neighboring ganglia. Although the nerves from this region supply mainly the lower portion of the esophagus they may have filaments uniting with branches from the cervical nerves to the crop. According to Greving (3),

such an arrangement occurs in the human esophagus. From this distribution an augmentor action on the crop as well as on the lower esophageal muscle might be expected. The branches from the stellate and neighboring ganglia are so small that we have found it impossible to dissect them all out satisfactorily and to stimulate them during life. We, therefore, tested their functional influence by stimulation of the spinal cord in the cervical and thoracic regions.

*Effects of spinal cord stimulation.* In decapitated pigeons with both autonomic nerves cut, electrical stimulation of the exposed cervical cord, which connects with the ganglionated chain in this region, gave a typical sympathetic response, namely, small contraction of the circular with simultaneous and more marked contraction of the longitudinal muscles. In the decapitated pigeons, the relay must have been over the sympathetic locally, since the superior cervical ganglion was cut off. Precisely the same result was obtained on stimulation of the thoracic cord in pigeons with the cervical cord and vagus nerves cut so that the impulses could spread over the lower sympathetic innervation. A similar result was obtained on thoracic cord stimulation in pigeons with only the vagus nerves cut and in those with the vagi cut and medulla destroyed. In these latter experiments, the impulses traversed the entire sympathetic innervation supplying the entire esophagus. The result was qualitatively the same, but the contractions of both muscles were smaller, when the thoracic cord was stimulated in pigeons with the cervical cord and both the vagi and sympathetic nerves cut low down. In this case the impulses traversed only the sympathetic filaments to the lower portion of the esophagus so that the number of impulses and fibers activated was smaller, and hence the contractions were smaller. Therefore, the sympathetic innervation to the lower portion of the esophagus seems to be functionally identical with that from the superior cervical ganglion. The cord stimulations in the thoracic region affected the heart in the usual manner, that is, with each stimulation there was a marked and immediate acceleration of the pulse. The results obtained indicate that both the cervical and thoracic cords activate the crop muscles and this could only be through the sympathetic nerves, since the vagi have their origin in the medulla and were eliminated in the tests. When the vagi were left intact together with the sympathetics and the thoracic cord stimulation was made, the muscular responses were identical with those of direct stimulation of the vagus trunks, namely, contraction of the circular with simultaneous relaxation of the longitudinal muscles. This was due to the greater tonic activity of the vagus mechanism. Corroborative evidence of the sympathetic innervation being responsible for the crop muscle contractions during cord stimulation was also obtained after atropine and nicotine. That is, the crop contractions still occurred after atropinization but not after complete

nicotinization, as might be expected. The results of thoracic cord stimulation before and after destruction of the medulla, vagus nerve section and after atropine and nicotine are illustrated in figure 7.

It is obvious that the results obtained with direct stimulation of the cord were identical with those of direct sympathetic nerve stimulation. Taken together they clearly indicate the absence of an inhibitory sympathetic influence on the crop muscles. This is quite different, therefore, from the sympathetic function to the stomach and intestine but, as pointed out above, it is consistent with expectations from the sources of supply which are the same as to the iris and to the heart, and from the effects of epinephrin, all of which are augmentor.

*Separate autonomic innervation of the crop muscles.* From the results of stimulation of the autonomic nerves just described, it is suggested that the nerves activate different muscles or mechanisms. That is, vagus nerve stimulation causes contraction of the circular with simultaneous relaxation of the longitudinal muscle (reciprocal action) followed by increased, or initiation of, peristaltic activity, while sympathetic nerve stimulation causes a contraction with increase in activity of the longitudinal muscle only. In other words, the vagus nerve activates the circular muscle and also the myenteric reflex, which is responsible for the initial longitudinal relaxation (discussed in the forepart of the paper) and later peristaltic activity; that is, the signal for the myenteric plexus would seem to be mediated through the contracting circular muscle. The sympathetic nerve activates mainly the longitudinal muscle, and apparently the relay must be direct and not through the myenteric plexuses since there is no initial reciprocal response of the muscles as is the case with vagus stimulation, or with pressure. From this it would appear that the vagus nerve supply primarily activates the circular muscle, and the sympathetic activates the longitudinal muscle. An almost perfect analogy to this arrangement exists in the iris, in which both the autonomic nerves are augmentory to the two sets of muscles, the parasympathetic nerve supplying the circular and the sympathetic the radial (longitudinal?) muscle fibers. This might be expected from the anatomical origin and distribution of the nerves to the esophagus. Obviously the mutual antagonism of the two sets of autonomic nerves in other portions of the alimentary canal does not hold in the crop, and presumably the entire esophagus, contrary to the supposition of Greving (3) regarding the human esophagus. From his anatomical evidence, Greving suggested that the ganglionic cells of the myenteric plexuses in the human esophagus belong to the type of sympathetic multipolar ganglionic cells, but our results with the crop suggest that the myenteric reflexes are activated through, if not connected with, the parasympathetic (vagus) nerve. Moreover, similarity of structure does not necessarily indicate similarity of function. To what extent

the sympathetic nerves influence or determine the muscular activity in the crop or esophagus under physiological conditions can not be said from our evidence. It would seem, however, that the vagus and myenteric reflex would play the principal rôle. This was suggested from the results on nerve degeneration.

*Results of nerve degeneration.* These have been previously described in our report on crop muscle responses in anaphylactic shock (1), and the results pertaining to certain drugs will be described in another paper. For the purpose of this report, the results showed that the vagus nerve function was more essential to crop function than the sympathetic nerves, for section and degeneration of the vagus nerves alone gave the same results as degeneration of both sets of nerves. That is, degeneration of both nerves caused a relaxation of the crop, complete inhibition of spontaneous peristalsis and early death. In fact, only 4 out of 32 pigeons used for the purpose survived long enough for satisfactory results on nerve degeneration. The median time of death was $9\frac{1}{2}$ days; and the period of nerve degeneration of the 4 pigeons used was from 11 to 28 days. The food remained packed in the crop and invariably was found decomposed. This meant that peristalsis was inhibited. In such pigeons, the remnants of nerve trunks found in the neck did not give crop responses on stimulation, but the muscles were still responsive, and on distention gave the usual reciprocal response of the two muscles indicating the preservation of some function in the myenteric plexuses. On the other hand, section and degeneration of the sympathetic nerves alone permitted the pigeons to live indefinitely, the crop evidently performing its usual functions. Hence, it appears that the sympathetic nerves are not indispensable to crop function in, and to the life of, pigeons. Thus the greater importance of the vagus nerves to the crop is indicated.

ASPHYXIA AND RELATED FACTORS. Asphyxia was found to be one of the most efficient stimuli to the crop muscles. Clamping the trachea of curarized pigeons resulted in prompt contraction of both the circular and longitudinal muscles. This lasted about 2 to 3 minutes and was followed by a permanent relaxation and paralysis. Occasionally a temporary post-mortem peristalsis continued, but as a rule the post-mortem contraction was followed by complete cessation of activity and responsiveness to electrical and drug stimuli. The same kind of contractions were observed in pigeons dying suddenly, though receiving artificial respiration at the time of death and post-mortem. Presumably a local asphyxia was here responsible for the effects. A sudden contraction occurring in a previously quiet or non-reactive crop is a fair warning of sudden cardiac stoppage and death. In routine experimentation with the crop it is advisable to observe the cardiac efficiency from time to time, a matter that is easily accomplished by means of a stethoscope, thereby avoiding misleading results.

Hemorrhages of varying amount, equivalent to $\frac{1}{20}$ of the blood volume or so, had no demonstrable effects on the crop muscles. The small oozing of blood which occurs from the axillary veins, used for introduction of hypodermic needles or cannulae for injections, is of no importance. Large hemorrhages resulting in death give the typical effects of asphyxia just described. The effects of blood pressure per se have not been specifically studied by us, but in the case of certain drugs, causing opposite changes in blood pressure (such as the rise of epinephrine and the fall of pilocarpine), the crop muscles were affected in the same direction, i.e., stimulated. Injections of 0.9 per cent sodium chloride solution up to 10 cc. per kilo had no demonstrable effects on the crop muscles.

RESPONSES OF EXCISED ESOPHAGEAL MUSCLE. Strips of excised esophagus were tried according to the Magnus method with the hope of ultimately using these in pharmacological experimentation, but the results were generally disappointing. Out of 100 strips from different portions of the esophagus only about 50 per cent were found active. We tried air and oxygen, Tyrode's, Locke's and Fleig's solution with and without the addition of blood, a Tyrode solution with different pH values and stretching and weighting the strips, cooling over night and using them fresh. It made very little difference what was done; a strip would show spontaneous contractions under any of the conditions tried but an adjacent strip might remain inactive. Ultimately, we settled upon ordinary Tyrode's solution with a pH of 7.8 which on aeration changes to 8.0 or 8.2, and containing 0.1 per cent dextrose, the same solution which is routinely used by most workers for excised mammalian organs. Strips of the lower portion of the esophagus showed the greatest activity, if they responded at all, while those of the crop were sluggish, but less so than those of the upper portion of the esophagus. Thus, there seemed to be a gradient of increasing activity from below upwards, but our results were so erratic that it is not permissible to draw any conclusions as to this feature. Strips of longitudinal muscle were generally more active than those of the circular muscle.

The strips which were active showed in general two types of activity; 1, slow, regular and rhythmic but small contractions, and 2, more rapid, powerful but irregular contractions. The slow type occurred about once every minute, while of the rapid there were 16 or 20 to the minute. The responses to cooling (from 42° to 32°C.) consisted of slow and incomplete, but sustained contractions indicating the usual stabilizing effect of cold. Heating (from 22 to 42°C.) caused sudden, marked contractions with rapid relaxation. Thus, the effects of cooling and of heating were the same as on smooth muscles generally.

The results obtained by us with excised pigeon esophagus, both as to the general type of contractions and the responses to cooling and heating,

confirm those of Prakken (12) with excised strips of cat esophagus composed of smooth muscle. The results with drugs will be described elsewhere.

## SUMMARY AND CONCLUSIONS

1. The anatomical arrangement and distribution of autonomic nerves to the crop (esophagus) in pigeons together with general physiological reactions of the circular and longitudinal muscles are described.

2. The reactions of the circular and longitudinal muscles were recorded under tension by a system which permitted records of their activity separately though simultaneously.

3. Under these conditions, spontaneous peristaltic contractions of both muscles occurred after decerebration and autonomic nerve section, but not in atropinized pigeons and after degeneration of both the vagus and sympathetic nerves, the latter resulting fatally. They occurred after sympathetic nerve degeneration and the pigeons lived.

4. The seat of the spontaneous peristalsis is therefore local and presumably mediated through the functional integrity of the vagus nerves and myenteric plexuses.

5. During spontaneous peristalsis the contractions of the circular muscle were marked, but slower and more regular than those of the longitudinal muscle, which at times was erratic and carried on independent contractions. Generally, there was a coördinated reciprocal action of the two muscles, so that as the circular muscle contracted the longitudinal muscle relaxed simultaneously and the relaxation was followed by several contractions.

6. A similar reciprocal response of the two muscles was demonstrated during vagus nerve stimulation, but not during sympathetic nerve stimulation.

7. A reciprocal relaxation of the longitudinal muscle occurred during distention of the living but not of the dead crop, showing that the relaxation was functional and not mechanical.

8. Since the reciprocal relaxation with distention pressures occurred in crops after section and degeneration of the autonomic nerves, but not after cocainization of the crop mucosa, its seat was local and apparently due to a reflex mediated through the myenteric nerve plexuses.

9. The two muscle layers of the crop appear to be activated by the autonomic nerves separately, for electrical stimulation of the vagus nerves causes a marked contraction of the circular muscle with simultaneous and variable relaxation of the longitudinal muscle the relaxation being mediated through the myenteric reflex, whereas stimulation of the sympathetic nerves causes mainly a marked contraction of the longitudinal muscle with very little or no change in the circular muscle.

10. The analogy to the muscular arrangement and the autonomic innervation of the iris, both functionally and anatomically, except for the myenteric plexuses, is striking.

11. The sympathetic innervation to the crop (esophagus) is augmentor in function as might be expected from the fact that anatomically the nerve supply is derived from the stellate (first thoracic) and the cervical (inferior, a chain of middle, and superior) ganglia whose functions are augmentor; epinephrine also acts as an augmentor for the crop muscles. This is contrary to certain speculations in the literature, which ascribe an inhibitory function to the sympathetic. The vagus function in the crop is similar to that of other portions of the alimentary tract.

12. Asphyxia causes contraction of the crop muscles, and is presumably the cause of post-mortem contractions, and of those accompanying marked hemorrhage.

13. Strips of the excised pigeon esophagus gave uncertain results but, in general, those of the lower portion were most active, those of the crop less active and those of the upper portion were least active. The spontaneous responses and those to heat and cold were typical of those of smooth muscle generally and similar to those of excised cat esophagus reported in the literature.

14. Since the sympathetic and vagus innervations to the human esophagus (Greving, 3) are practically identical with those of the pigeon esophagus, their functions are presumably also the same (probably also in the cat), but in other species in which the esophagus is composed mostly of striated muscle the innervation and the functional responses may be different.[2]

[2] Using a balloon in the cardia (circular muscle only?) of cats with esophageal and gastric fistulae, Professor Carlson and associates (This Journal, 1922, lxi: 14) demonstrated that stimulation of the vagi or sympathetic nerves caused contraction of this portion of the organ, if in low tone, and relaxation, if in high tone; in dogs sympathetic stimulation gave only augmentation; and in rabbits, only inhibition. In a monkey, stimulation of the peripheral ends of the splanchnics or of the vagi gave contraction (Carlson and Litt: This Journal, 1923, lxv: 534). On the other hand, Carlson and Luckhardt (This Journal, 1921, lvii: 299) found no evidences of sympathetic innervation to the turtle esophagus, or of inhibitory, sympathetic fibers to the frog esophagus, although epinephrin stimulated the former and inhibited the latter. These papers discuss the older literature on esophageal innervation.

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# THE EFFECTS OF SELECTIVE SOLAR IRRADIATION ON THE GROWTH AND DEVELOPMENT OF CHICKS

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The chicks used (5) in the investigations on the effects of selective solar irradiation on the development of the parathyroids were employed also in an endeavor to ascertain whether or not the presence or absence of the shorter wavelengths of solar irradiation, as well as the practical exclusion of longer or shorter portions of the visible spectrum, had any influence in their growth.

Approximately 250 chicks about a week old, of equal grade and the same stock, were divided, without any attempt at selection, into eight groups of equal numbers and placed in an experimental house provided with windows about 10 feet square of transparent medium. The four filters used were: cathedral or hammered vitaglass, ordinary window glass, blue glass (no. 56 Pittsburgh), and amber glass (no. 48 Pittsburgh). The chicks in each compartment were divided equally in two sections, each compartment being divided by a partition of chicken-netting. The chicks in one portion of each of the four compartments were fed on a standard Wisconsin all-mash ration, consisting of 80 pounds yellow corn meal, 20 pounds shorts, 5 pounds bone meal, 5 pounds limestone grit, 1 pound salt; skimmed milk was given freely with no water the first two weeks. To the feed of the chicks in the second part of each compartment was added 2 per cent (by weight) of Squibb's cod-liver oil. The hours of feeding and the quantities of food given were kept constant. Temperature and humidity varied from day to day but were uniform each day in all compartments. The controllable factors which were varied consisted of the character of irradiation, and the presence or absence of 2 per cent of cod-liver oil in the rations.

Spectrograms of the transmission of the four light filters used are shown in figure 1. Spectrophotometric curves of the percentage transmission of light of various wavelengths, both in the visible and ultraviolet regions, are to be found in the paper by Higgins and Sheard (5). The essentials regarding the differences in the character of the radiant energy transmitted are briefly: 1, vitaglass transmits about 50 to 60 per cent of the solar ultraviolet irradiation as well as a high percentage (85 to 90 per cent) of

all visible light; 2, ordinary window glass transmits to about 310 millimicrons, hence cutting off the shorter ultraviolet rays of sunshine as well as decreasing the percentage transmission at longer wavelengths in the ultraviolet portion; 3, blue glass (no. 56 Pittsburgh) absorbs quite completely (to within 20 per cent) from 680 millimicrons to 520 millimicrons, but transmits a maximal amount of the short visible blue-violet portion and has a slightly greater percentage transmission in the ultraviolet region than does ordinary window glass, and 4, amber (no. 48 Pittsburgh) transmits only the longer visible wavelengths (500 to 700 millimicrons).

EXPERIMENTAL DATA. The growth of the chicks was estimated by obtaining the average weight of the same ten chicks (banded) from each of the eight compartments. The results for the first eighty days are shown in figures 2 to 5 inclusive. In each figure, the upper curve (curve *1*) shows the relationship between the average weight (ounces) and the time (in days) since the beginning of the experiment when 2 per cent cod-liver oil was

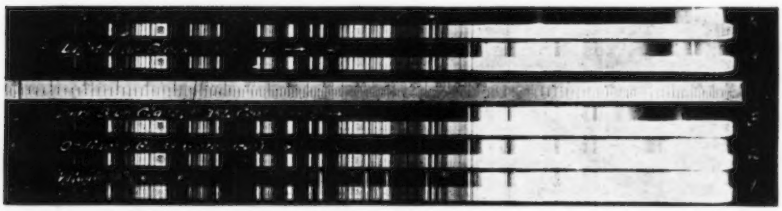

Fig. 1. Spectograms showing the limits of transmission of the various glass filters used. The lower half of each set of spectrograms shows the spectrum of the source only.

added to the otherwise standard diet. Curve *2*, in each instance, shows the weight-time relationships when a standard diet only was fed.

At the end of the period of eighty days it became necessary to kill, from time to time, some of the banded chicks from each group. The weighings were discontinued, therefore, until six months had elapsed. At that time not less than three nor more than five of the original ten banded chicks in each group remained. The average weight of chicks, kept behind the specified filter and under the diet indicated, at the end of six months is shown in table 1.

*Comment.* All the curves of figures 2 to 5 show that the average weight on any selected day is slightly greater when cod-liver oil is added to the diet even under other conditions, present in the summer, which are favorable to growth. The least difference in this respect exists in the case of chicks kept constantly behind either vitaglass or blue glass. The difference between the curves of growth is more marked in the case of ordinary window glass and is pronouncedly increased under amber glass.

If figures 2, 3 and 4 are superimposed it will be found that, in each case, curve *1* falls almost exactly on curve *1*. The same is true of the curves marked *2*. In the case of the results under amber glass (fig. 5), however, it will be found that the rate of growth, as evidenced by weight, when cod-

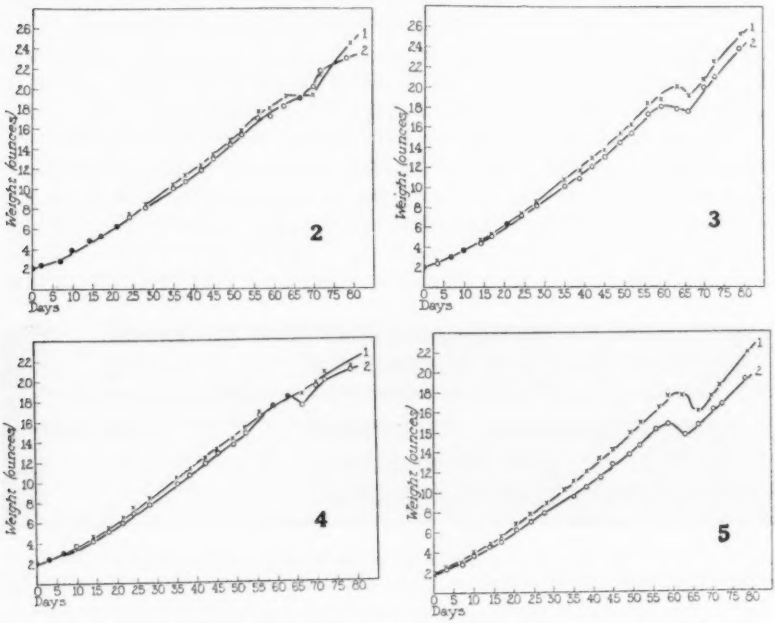


Fig. 2. Curves show the average weight of a selected group of chicks grown constantly under vitaglass. Curve *1*, Wisconsin all-mash ration with 2 per cent cod-liver oil; curve *2*, Wisconsin all-mash ration only.

Fig. 3. Curves show the average weight of a selected group of chicks grown constantly under ordinary window glass. Curve *1*, Wisconsin all-mash ration with 2 per cent cod-liver oil; curve *2*, Wisconsin all-mash ration only.

Fig. 4. Curves show the average weight of a selected group of chicks grown constantly under blue glass (no. 56 Pittsburgh). Curve *1*, Wisconsin all-mash ration with 2 per cent cod-liver oil; curve *2*, Wisconsin all-mash ration only.

Fig. 5. Curves show the average weight of a selected group of chicks grown constantly under amber glass (no. 48 Pittsburgh). Curve *1*, Wisconsin all-mash ration with 2 per cent cod-liver oil; curve *2*, Wisconsin all-mash ration only.

liver oil is present in the diet, is slightly greater than in the corresponding curves (marked *1*) obtained under vitaglass, ordinary glass and blue glass. Also, the rate of growth with the standard diet alone is appreciably decreased under amber glass as compared with the results obtained under the other light filters.

All the curves of figures 2 to 5 either clearly show or indicate the presence of a decrease in weight during the period between the sixtieth and seventieth days. Just prior to and during the first portion of this period of summer months the temperature and relative humidity were high. The most marked effects are shown under amber glass. Since the conditions of temperature, humidity, ventilation and the like were identical in all compartments, and since the minimal effects as to loss of weight were experienced under filters which transmitted ultraviolet to wavelength 310 millimicrons or lower, we may postulate that the presence of such solar ultraviolet irradiation has been a factor in the building-up, in the growing chick, of a resistance to or a combatant of untoward physiologic conditions. While cod-liver oil seems to have, in large part, compensated for the absence of vitamine D, it does not appear to be exactly identical with it.

TABLE 1

*Average weight of chicks kept under various selective light filters six months after hatching*

| FILTER | RATION | AVERAGE WEIGHT |
|---|---|---|
| | | ounces |
| Vitaglass | Standard with 2 per cent cod-liver oil | 54.7 |
| | Standard | 55.3 |
| Ordinary glass | Standard with 2 per cent cod-liver oil | 54 |
| | Standard | 50 |
| Blue (no. 56 Pittsburgh) | Standard with 2 per cent cod-liver oil | 55.7 |
| | Standard | 45.0 |
| Amber (no. 48 Pittsburgh) | Standard with 2 per cent cod-liver oil | 56.7 |
| | Standard | 39.3 |

This postulate is also supported by experimentation on the part of other investigators. Or possibly it should be said that the presence of ultraviolet rays of short wavelength (290–330 millimicrons) is an added factor in overcoming various degenerative tendencies. There is no reason for believing that the loss of fat, possibly developed by the feeding of cod-liver oil, should be the factor involved, since a half of each of the four groups received this substance and in equal quantity.

Table 1 shows that in the autumn (October) the average weight was nearly the same under each of the four filters, provided 2 per cent (by weight) of cod-liver oil was added to a good standard ration. But little difference exists in the matter of average weight under vitaglass or ordinary glass whether or not cod-liver oil is fed. From these data we may conclude that there is normal metabolism during the summer months under ordinary window glass. This would indicate that window glass, while it does not

transmit wavelengths of less than about 310 millimicrons, still transmits sufficient ultraviolet and visible solar energy during the summer, when optimal energy conditions exist, to allow normal growth to take place.

In the case of the average weight of the chicks under blue and amber glass, however, there is definite proof that normal growth and development have not taken place in the absence of cod-liver oil, whereas they have occurred when the ration contains this substance. There was a 20 per cent decrease in weight under blue glass and a 30 per cent decrease in weight under amber glass if no cod-liver oil was fed. Furthermore, in the case of feeding on a standard diet, this underdevelopment, as evidenced by weight, occurs when a considerable amount of ultraviolet light is transmitted with the practical exclusion of the longer visual rays (blue glass) and vice versa (amber glass). We are forced, therefore, to the conclusion, even in the face of evidence to the contrary on the part of other investigators, that the presence of both the longer and shorter (ultraviolet) wavelengths of sunlight is essential to normal growth. We are led also to conclude that cod-liver oil is able largely, if not wholly, to compensate for the absence of either the longer or shorter wavelengths of solar irradiation. These results further emphasize the rôle which the parathyroids play in the growth and development of chicks (5). Our results have shown that hyperplasia of these organs occurs under blue and amber filters and that this hyperplasia is minimized when cod-liver oil is administered. We may postulate that hyperplastic parathyroids develop in an attempted rectification of hypofunctioning through an increase in the size of that which we may call the "factory," the parathyroids, in order to produce as nearly normal development and metabolism as is possible. It would seem as though the normal unit lacks sufficient motive power to produce the requisite metabolism and that, in the initial stages, normal growth and development are accomplished through a multiplicity of the functioning units.

The results of our observations on the weights of chicks under the experimental regimen described are not in accord with the observations of others, particularly those of Bovie (1) and his colleagues. In the summer of 1924, at the University of Maine, they observed a clutch of 250 chicks that were about a week old. In this respect, their experiments and ours are similar. They divided these into six groups, placed them in a greenhouse and treated them variously, the essential difference being that each group, confined to the greenhouse and fed a given diet, had a counterpart with either outdoor sunshine or irradiation by a quartz mercury lamp. The groups were fed regular food, regular food and green stuff, and regular food and cod-liver oil. After separately grouping the weights of the treated chicks and controls, these authors exhibit a set of curves showing the percentage increase of the treated over the control chicks. Bovie (2) writes: "It will be seen that at the end of the sixty-fifth day the total weight of the chickens

receiving sunlight only through the greenhouse roof is about one-half of the total weight of all the chickens exposed to outdoor sunlight or the light from the quartz mercury vapor lamp." As previously stated, our results under vitaglass (which transmits to wavelengths shorter than are contained in outdoor sunshine, approximately 50 to 60 per cent of the total ultraviolet content of such light and an average of 25 per cent of the so-called vital or health-giving rays) shows slight difference in average weight when compared, during the first sixty-day period, with the results under ordinary glass and blue glass, which transmit considerable ultraviolet (as low as 310 millimicrons), or even under amber glass which transmits only the longer visual rays. We have not included in our presentation any data relative to chicks fed on a standard diet and allowed to run out of doors. We believe that no logical conclusion could be drawn by reason of lack of control, such as exercise, outdoor temperature, extraneous foods and the like. However, a group of chicks of like age and comparable stock allowed to run out of doors were compared, as to weight, with an equal number of chicks from the compartment carrying vitaglass, and the two groups were practically the same, the difference being slightly in favor of the outdoor chicks.

This disparity of experimental data and consequent conclusions may be due either to differences in quality of stock used, the character of the ration fed or to both. The stock used by us was graded 2 (or B) by the poultry men and the chicks were as nearly biologically equal as is likely to occur. We cannot pass by the opportunity to comment on the necessity of high quality and uniform quantity of animals used in research. Mixed quality or inferior stock will lead unquestionably to varying conclusions in the hands of different investigators. Inferior stock will succumb to circumstances which will have no effect on superior stock. We have in mind the repetition of our experiments using low-grade stock. The only objection is that we shall be in doubt as to what low-grade consists in.

The matter of ration is evidently very important. A well-known investigator in the effects of ultraviolet irradiation on animals, after viewing the experimental chicks, expressed his opinion that they were as fine indoor chicks as he had ever seen. The question of ration was discussed. Our ration was high in mineral content. We may well question what experimental data would have indicated had we used a diet low in content of minerals, such as calcium. For an efficient factory, working on a reduced supply of raw material, might produce less than an inefficiently operating plant with a surplus of raw material. Determinations on the blood calcium and phosphorus made biweekly during these experiments showed that the calcium was about 12 mgm. for each 100 cc. of serum and the phosphorus about 6 mgm. for each 100 cc. of serum during the first two months of observation, irrespective of the character of the light filter. In

the autumn, however, changes occurred which are now under investigation. Furthermore, no gross, roentgen-ray or microscopic evidence of rickets occurred during the first sixty days in any of the chicks.

The question naturally arises as to whether the total quantity of energy in the solar irradiation transmitted by the various filters is a crucial factor. So far as distribution (infrared, visible and ultraviolet) is concerned, we believe, from our recent measurements and those formerly made (7), (8), as well as those by Coblentz (3), and Coblentz, Dorcas and Hughes (4), that the percentage transmission of infrared by the various filters used cannot be a factor, since this percentage transmission is comparable in all cases (table 2).

Neither does it appear that the total quantity of visible energy transmitted by those filters should be a determining factor in either hyperplasia of the parathyroid glands or underdevelopment of the animal as evidenced by loss of weight. For amber and blue glass transmit, respectively,

TABLE 2

*Percentage transmission of the filters used in the case of vital rays, total ultraviolet, visible and infrared radiations*

| SPECTRAL REGION | TRANSMISSION | | | |
|---|---|---|---|---|
| | Vitaglass | Ordinary window glass | Blue No. 56 | Amber No. 48 |
| | *per cent* | *per cent* | *per cent* | *per cent* |
| Vital rays (280–320 mμ) | 30 | 2 | 3 | 0 |
| Ultraviolet (280–400 mμ) | 70 | 35 | 43 | 0 |
| Visible (400–750 mμ) | 90 | 85 | 40 | 30 |
| Infrared (750–4250 mμ) | 70 | 70 | 60 | 50 |

practically only the longer (red, orange, yellow) and the shorter waves (green, blue, violet) of the visible spectrum and that in comparable percentages. Also the green-violet region of solar irradiation amounts to about 16 per cent and the red-yellow region to about 22 per cent, hence as far as quantity of energy in the visible spectrum (400–750 millimicrons) is concerned, there is practical equality and a sufficiency. But there is obviously a marked difference in quality (wavelength).

Two per cent of so-called vital rays is transmitted by the ordinary window glass used, 3 per cent by the blue glass and none by the amber. And yet practically as great a loss of weight occurs under blue glass as under amber glass. There is practically no difference in average weight under window glass or vitaglass, yet the first filter transmits only 2 per cent of vital rays, whereas vitaglass transmits 30 per cent.

If we accept the statement of Kimball and Hand (6) that we receive only about 10 per cent of full sunshine through southern exposures (as was the

case in our experiments) and the statement of Coblentz that the radiant flux of sunlight at sea level is 0.088 watt for each square centimeter (1.25 gram-calorie), of which 2 per cent is vital rays and about 7 per cent is ultraviolet irradiation, calculations show that the energy equivalent of the vital rays transmitted by ordinary glass or blue glass is 0.003 gram-calorie for each square centimeter each minute. The energy of the vital rays transmitted by vitaglass is about fifteen times that of ordinary window glass, hence the energy equivalent of the transmitted short-wave ultraviolet solar rays is 0.045 gram-calorie for each square centimeter each minute. Since the development of chicks under vitaglass appears to be wholly comparable to that under full sunlight, we may accept the tentatively suggested value of 0.005 gram-calorie for each square centimeter each minute as the unit of dosage which, when applied over a sufficient period of time, will prevent malnutrition and deficiency diseases. The question obviously arises as to the total daily dosage in order to produce and keep normalcy.

We do not desire at present to venture any explanations as to why the presence of both the longer visible and ultraviolet irradiations seems to be essential for normal growth and development. So far as we know, however, there is no evidence to prove that ultraviolet light of a given range of wavelength is capable, in and of itself, of preventing rickets or nutritional deficiencies. For quartz mercury vapor lamps, as used therapeutically, and with an energy input of 500 to 600 watts, are calculated to radiate about 30 per cent infrared, 35 per cent visible and 35 per cent ultraviolet, and carbon arcs of the type used for treatment emit 75 per cent infrared, 15 per cent visible and 10 per cent of ultraviolet.

## SUMMARY AND CONCLUSIONS

Under the experimental conditions, grade of stock and ration used in our investigations we believe that:

1. The rate of growth of young chicks, as evidenced by weight, is either slightly or not at all affected during the first two months of life when continuously housed behind selective filters which permit of the passage of maximal or minimal ultraviolet irradiation, or maximal or minimal quantity of energy of longer visual wavelengths.

2. During the first sixty days the rate of growth as evidenced by weight is slightly greater when 2 per cent by weight of cod-liver oil is added to the standard diet, irrespective of the light filter.

3. At the end of a six months' period, the average weight of chicks fed on the standard diet and 2 per cent cod-liver oil is practically the same, irrespective of the light filter used (vitaglass, ordinary window glass, blue glass, and amber glass). This supports the hypothesis that cod-liver oil

is able to induce normal growth irrespective of the presence or absence of any portion of either ultraviolet or visible solar energy.

4. A decided decrease in average weight was found at the end of six months in the case of chicks housed behind blue and amber glass and fed on a ration which did not contain cod-liver oil.

5. Evidence is presented to support the statement that normal growth and development are dependent on both the ultraviolet and visible portions of solar irradiation, all other factors remaining constant so far as is known.

6. Our results, so far as they are applicable, do not substantiate the observations of other investigators relative to the marked differences in weight of outdoor chicks as compared with those confined to a greenhouse.

7. The importance of selection of stock and the question of the character of ration, particularly the percentage mineral content, are discussed.

8. The average weights under various light filters are correlated with the data relative to hyperplasia or normalcy of the parathyroids. Hyperplasia of the parathyroids, as evidenced under amber and blue glass, substantiates the hypothesis that this overdevelopment occurs in the interest of maintaining normal growth and development. Ultimately, however, this is not adequate and subnormal weight and signs of rickets occur.

9. Experimental data on the quality and quantity of energy in solar radiation transmitted by the various filters and calculations made therefrom indicate that an energy-equivalent of the short or vital rays (290 to 320 millimicrons) of 0.045 gram-calorie for each square centimeter each minute is ample to produce normal development.

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# THE EFFECTS OF SELECTIVE SOLAR IRRADIATION ON THE PARATHYROID GLANDS OF CHICKS

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Hyperplasia of the parathyroid glands is a frequent observation among students of those diseases involving a disturbance of the normal calcium and phosphorus metabolism. Erdheim (1914) in extensive studies on rats, noted marked hyperplasia of the parathyroids of animals with spontaneous rickets. Pappenheimer and Minor (1921) in studying these glands in cases of rickets in human beings recognized marked increase in the number of cells. Luce (1923) concluded that the calcium content of the diet bears a direct relationship to the size of the gland. In rats maintained on a diet low in calcium for twelve weeks, the parathyroids were many times larger than normal.

Fowls have not been used extensively in studies of these glands. Doyle (1926) recognized greatly enlarged glands in rachitic chickens, and more recently Nonidez and Goodale (1927) have shown that the parathyroids of chicks become enlarged when they are kept from direct sunlight on a ration poor in the antirachitic vitamine.

Since parathyroid hyperplasia seems definitely correlated with calcium insufficiency and since calcium metabolism is in some way dependent on vitamine D, present either in the diet or in the shorter wavelengths of sunlight, we are interested to know to what extent selective solar irradiation might modify the size of these glands in chicks maintained on identical diets in which the calcium content was adequate. Accordingly four convenient pens were constructed, and screened on their southern exposures by amber, blue and ordinary glass, and vitaglass, thus transmitting variable portions of the sun's spectrum. Each of these four pens was divided by a median partition into two smaller pens, so that four pairs of compartments, each pair illumined by the same light, formed the physical equipment for the experiment. The filters used throughout the experiment were cathedral amber (no. 48 Pittsburgh), cathedral blue (no. 56 Pittsburgh), cathedral window and cathedral vitaglass. Each pane was from 2.8 to 3 mm. thick.

Various spectroscopic and spectrophotometric tests were made to deter-

mine the transmission of rays by these filters in both the visible and the ultraviolet portions of the spectrum. Roughly speaking, the transmission of infrared irradiation of various wavelengths by all of these filters was practically equal; hence the possibility of variations in such transmission having any effect on the outcome of the study is eliminated. Spectrophotometric determinations of the percentage transmission of the various wavelengths by the filters are plotted in figure 1. The curves show that vitaglass permits the maximal transmission of ultraviolet irradiation, followed by the blue glass, ordinary glass and the amber glass, in the order of decreased transmission for the ultraviolet portion of the spectrum. The vitaglass transmitted to 272 millimicrons, the ordinary glass to about 309 millimicrons, with a faint trace to 303 millimicrons, the blue glass to 303

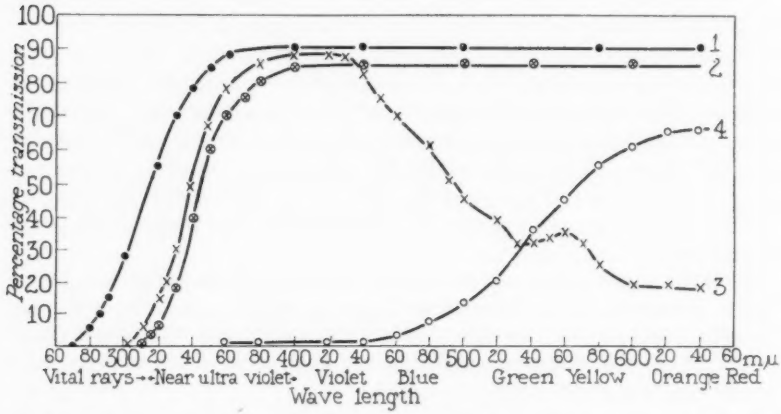



**Fig. 1. Spectrophotometric determinations of the percentage transmission of various wavelengths by vitaglass (curve *1*), ordinary window glass (curve *2*), blue glass (no. 56 Pittsburgh, curve *3*) and amber glass (no. 48 Pittsburgh, curve *4*).**

millimicrons, and the amber gave a very weak spectral transmission below 550 millimicrons. Photographic tests, however, with sufficiently long exposures showed that the transmission through the amber reached 345 millimicrons, but that the transmission in the ultraviolet region (wavelengths less than 400 millimicrons) is less than 1 per cent (fig. 1).

The basic diet throughout the experiment was the Wisconsin all-mash ration (see first reference). The ration, without the cod-liver oil, was provided for the chicks in one of each pair of pens under the four filters, while the ration with the cod-liver oil was fed the chicks in the adjacent pen under each filter. The chicks were placed in the pens April 22, when three days old, and the experiment continued until October 20, six months later. The weight of the chicks was noted each week during the

first half of the experiment. These data, with certain others incident to the experiment, are presented elsewhere in This JOURNAL (Sheard and Higgins). In this paper we shall present only those observations that concern the response of the parathyroid glands to the different environmental and food factors. Certain chicks in each pen were killed after two weeks, three weeks, one month, two months and three months. The region of the thyroid and parathyroids was carefully exposed and then, to avoid disturbance of the morphologic relations of these organs, the entire anterior half of the animal was fixed in Bowin's fluid. The entire area, including the thymus, lymph nodes, thyroid and parathyroid glands, was sectioned and stained, so that any accessory parathyroid tissue, so prevalent in the bird group, could be readily identified. Reconstructions in wax were made of the parathyroid glands on the left side of certain chicks in each pen at one month and three months of age and in this way some idea of the comparative volume of these glands could be determined. It is true that there may be some discrepancy in observations owing to the fact that data concerning the parathyroids of one side only were secured, but gross observations made on the glands of both sides at the time of fixation showed similar conditions. To determine the relative size of the parathyroids of the chicks at the end of the experiment, the glands were fixed in the same way, dehydrated and weighed moist, the excess fluid drained by filter paper.

All chicks thrived on the ration provided. The usual loss attendant on the culture of very young chicks was not encountered, even in the pens where the chicks were deprived of cod-liver oil and direct sunlight. Throughout the entire experiment only six chicks were lost; the cause was entirely unrelated to the particular experiment. The growth curves in these various groups, presented elsewhere in This JOURNAL (Sheard and Higgins), showed but slight differences. One is impressed with the relative similarity of growth of the chicks under the amber glass with that under vitaglass. Although the chicks in all of the pens appeared entirely normal throughout the earlier months of the experiment, a marked difference in the size of the parathyroid glands was early recognized in the chicks that were killed for study. Differential blood counts and determinations of the blood calcium and phosphorus levels, made frequently during the investigation, or roentgenograms, failed to reveal even any slight organic change that may have been in progress; and yet the parathyroid glands of chicks growing under amber and blue glass were fast becoming hyperplastic, a condition more pronounced in the chicks whose diet did not contain 2 per cent cod-liver oil.

In the chick the parathyroid tissue is usually massed into two small bodies which lie on either side at the posterior pole of the thyroid gland closely adherent to the carotid artery. Occasionally these two bodies

appear as a single fused structure, and often parathyroid tissue is found at the anterior pole of the thyroid, or imbedded within portions of the thymus gland. The parathyroid is an epithelial structure of endodermal origin and in young chicks it is composed of columns of cells separated from each other by extensive connective-tissue stroma. The cells are large, and comprise a reticulate cytoplasmic structure with elliptical nuclei containing numerous nucleoli. Mitotic figures are abundant in these cords of parathyroid cells in growing chicks. After three weeks of experimental observation, however, a differential growth of the glands in these chicks under the various filters is manifest. In each pen the chicks fed the ration including cod-liver oil had glands considerably smaller than the chicks maintained on the basic ration alone. Furthermore, the parathyroids of the chicks growing under vitaglass were smaller than those of chicks under ordinary, blue, or amber glass. This increase in volume is apparently due to hyperplasia, for the cells comprising the glands in the chicks growing under the amber and blue glass were no larger than those of glands growing under vitaglass, although the volume of parathyroid tissue in birds growing under the longer wavelengths of sunlight is fully one-third more at three weeks of age than that of birds in the vitaglass group. Occasional mitotic figures were found, but not oftener than in the smaller gland. Hyperemia, however, seems characteristic of these larger glands and the connective-tissue stroma separating the cords of cells is more extensive. The nuclear cytoplasmic ratio in the enlarged gland is not essentially different from that of the normal and there is no evidence that the functional cytoplasmic activity of these cells has been impaired by the marked increase in their number. Hyperplasia characterized the glands of all chicks that were denied the lesser wavelengths of sunlight, as was provided through the vitaglass filter, but it was less pronounced in the chicks that were fed the cod-liver oil. The vitamine D, present in the oil, appears then to compensate partially for the absence of direct sunlight, at least as far as the size of the parathyroids is concerned.

Leg weakness, such as usually develops in chicks withheld from direct sunlight, did not appear in our flock for five months after the beginning of the experiment. Neither did it occur in the chicks that lacked the cod-liver oil portion in their diet even under the amber, blue or ordinary glass. To be sure, the diet employed was unusually high grade and contained a slight portion of vitamine D even without the cod-liver oil; for we were not attempting to produce rickets, but rather to observe the glandular changes consequent to a satisfactory diet, varying only the light factor of the environment.

Under such conditions, the organism immediately responds to the lack of the lesser wavelengths of sunlight by the rapid increase in the number of parathyroid cells. This is especially true in the amber and in the blue pens,

although parathyroids of chicks screened from the sun by ordinary glass are considerably larger than those grown under the solar radiant energy transmitted by vitaglass. The cells in the hyperplastic glands are not essentially different from those of the normal parathyroid and one concludes that the cells are functioning and that secretory activity is accentuated so that the hormonal output may be doubled or tripled as the case may be. At the end of a month a chick grown in the blue pen without cod-liver oil

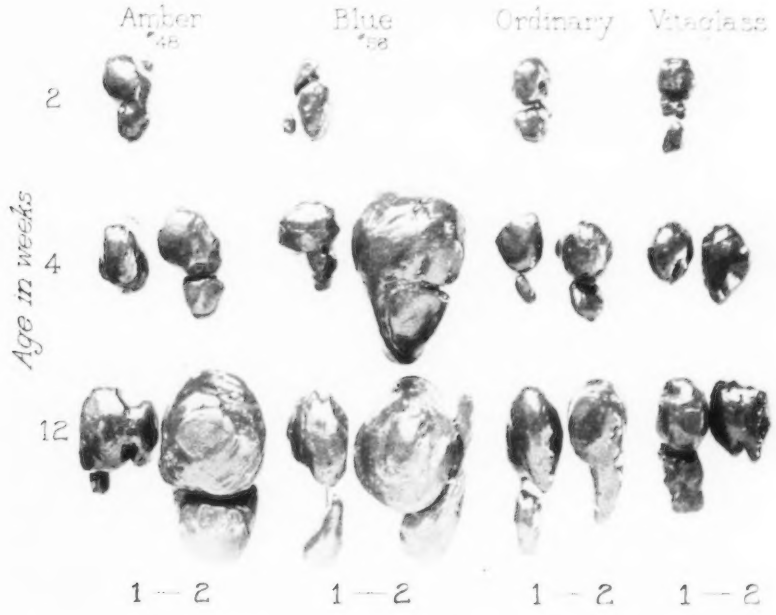



Fig. 2. A series of models of the parathyroid glands of chicks grown under the various filters with and without cod-liver oil. (× 50). The numeral 1 signifies that 2 per cent cod-liver oil was added to the standard diet. In 2, the standard Wisconsin ration only was fed.

was found to possess a fused parathyroid gland on the left side nine times the size of the fused gland on the same side of a chick grown under vitaglass on exactly the same diet. This was unusually large, but the chick appeared normal and healthy in every way; the gland was sectioned and reconstructed for comparative purposes (fig. 2).

During the first three weeks of the experiment, hyperplasia and hyperemia were the only histologic characteristics that differentiate the glands grown in the absence of the shorter wavelengths of sunlight (fig. 3). Here

it should be noted that some hyperplasia was manifest in the glands of the chicks grown under the shorter wavelengths of sunlight but on a diet devoid of cod-liver oil. This was quite characteristic throughout the glands that were studied and it would appear that the vitamine D of sunlight is not entirely adequate for the needs of the organism, if we are to judge by the hyperplasia induced within the parathyroids whenever vitamine D is missing in both diet and light.

At the conclusion of the third week of experimental observation regressive changes within the enlarged glands became coincident with the hyperplasia. Cells appeared to be produced at the periphery of the gland, while at the same time destructive changes went on within. These changes were especially marked in the glands of chicks growing under the amber and the blue glass, but less pronounced under the ordinary glass. Regressive changes were apparent, but far less pronounced in the glands of chicks maintained on the basic diet with cod-liver oil.

This regression was first recognized by a marked increase in the connective-tissue stroma, followed by breaking-down of the epithelial cords of cells (fig. 4). Our observations here substantiate largely those of Nonidez and Goodale (1927) who recognized shrinking of the epithelial cords in parathyroid glands of chicks after three months of continuous lack of direct sunlight. In the same section of glands undergoing regression one may see normal cords of cells with normal nuclei and cytoplasmic structures at the periphery, together with disintegrated cords, composed of shrunken cells with small nuclei, interspersed with large areas of connective-tissue stroma. Oval cells with spindle-shaped nuclei, probably fibroblasts, abound throughout this stroma.

With these regressive changes involving destruction of the cords and shrinking of the cells in the hyperplastic glands we recognized two types of cystic degenerative processes. These changes involve the formation of two

Fig. 3. A parathyroid gland in early hyperplasia, showing extensive hyperemia. (× 560)

Fig. 4. Parathyroid gland of a chick three weeks old, showing early regression and beginning cyst formation. (× 168)

Fig. 5. A portion of the columnar epithelial wall of a cyst within the parathyroid of a chick grown under amber glass. (× 960)

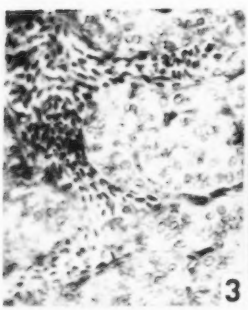
Fig. 6. Section through the entire cyst within the parathyroid gland of a chick grown under amber glass. (× 560)

Fig. 7. A portion of the flattened epithelial wall of a cyst within the parathyroid of a chick grown under blue glass. (× 960)

Fig. 8. Section through the entire cyst and parathyroid gland of a chick grown under blue glass.

Fig. 9. Section through the median plane of a parathyroid gland of a chick three months old, grown under blue glass. (× 54.4)

Fig. 10. Section through the median plane of a parathyroid gland of a chick, three months old, grown under vitaglass. (× 54.4)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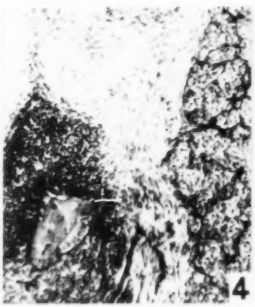
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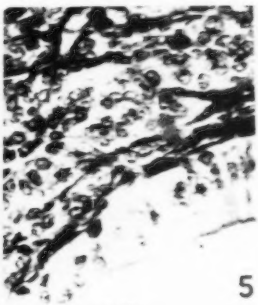
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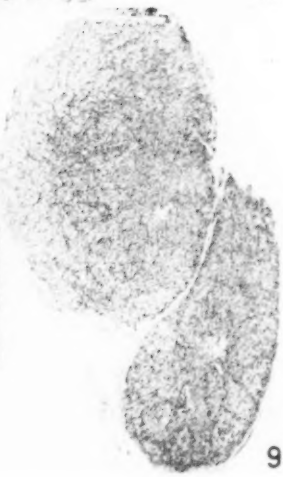
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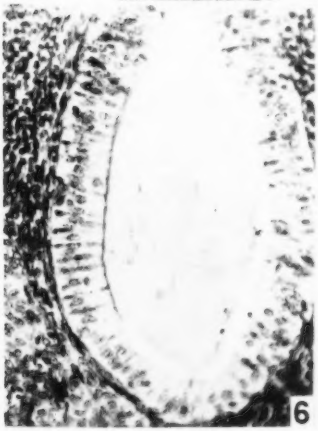
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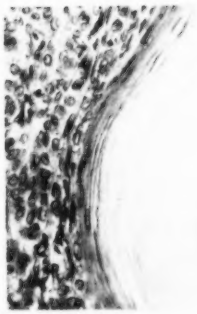
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distinct types of cysts within the gland. One of these consists of a wall composed of high columnar epithelium, supported on a thin basement membrane (fig. 5), while the other is walled off from the surrounding parathyroid tissue by a series of flattened cells concentrically arranged (fig. 7). We have not observed these two types of cysts coexistent in the same gland, and we have only found them in animals grown under light devoid of the lesser wavelengths of sunlight and usually more extensive if the ration is devoid of cod-liver oil, although occasional exceptions are to be noted. For example, figure 4 shows a section of a parathyroid gland with a mucous cyst walled off by high columnar epithelium, taken from a gland of a chick growing under the amber glass on the basic ration including the oil. This chick was externally normal in every way and there was evidently sufficient functioning parathyroid tissue to meet the needs of the organism. Doyle (1925) has recognized similar degenerative changes in chickens subnormal in ways other than rickets.

The origin of so typical a columnar epithelium as surrounds certain of these cysts is not clear. Apparently Nonidez and Goodale did not encounter these pathologic changes in their study, for they did not mention them. As these cysts first appear there is no evidence of such epithelial organization, but the sequence in the complete encystment appears somewhat as follows: As the cords disintegrate numerous cells appear with large droplets of mucus throughout their cytoplasm. These mucous masses stain reddish yellow with hematoxylin and eosin. The mucus is excreted from certain adjoining cells into an adjacent intercellular area forming thereby a nucleus of the future cyst. Other cells contribute their contents to the mass and these usually remain closely packed around the periphery of the structure, but occasionally these same cells become enmeshed in the mucus and so remain as the cyst increases in size. Gradually as the cyst increases the columnar epithelial capsule is laid down. The high columnar cells in the final cyst formation appear to be parathyroid cells which have broken away from their normal cord formation to wall off this cyst from the rest of the gland. In some sections masses of these cells appear to encroach on the region of the cyst, reorganize, assume a columnar shape, and form this very definite epithelioid structure. Thus when finally formed the mucous cysts are completely walled off from the surrounding parathyroid tissue by an epithelioid structure composed of high columnar cells closely packed together with elongated ellipsoid nuclei (fig. 6). These cells rest on a basement membrane composed of a layer of greatly compressed cells. In cross dimension the cysts have frequently been observed to equal one-fourth the area of the parathyroid gland itself.

The other type of cyst which we have encountered in the regressive processes of these enlarged parathyroids corresponds quite closely with the concentric masses of keratin that Nonidez and Goodale uncovered in the

parathyroids of chickens in tetanic seizures. We have recognized this condition in chicks one month old, growing under the blue glass, without cod-liver oil. Even at this age the cyst may involve a considerable portion of the gland, and yet sufficient tissue remain to provide adequate function, since no organic defect could be detected at the time of death. When these structures first appear, small groups of cells are massed together in what appears to be a gelatinous matrix. These cells are without cell boundaries and the nuclei are exceedingly large, vesicular and contain large deeply staining nucleoli. The origin of the matrix is not clear, but it probably arises as a secretion within these particular cells. It is not at all certain that it is keratin but some mucoid material, a by-product of the degenerative changes. As degeneration progresses the original mucoid mass increases in volume by the successive accretion of layers around the periphery by degenerative cells of the cords and thus the cyst in cross section appears as a series of concentric rings (fig. 8). Many of the nuclei of the cells which contribute to the formation of the cyst are taken up and appear in concentric layers of the gelatinous mass. Separate centers of cyst formation may become confluent, thus forming a larger unit, but the configurations of each center of origin remain evident even in the final fused structure.

Discussion. A study of the series of reconstructions (fig. 2) shows that there is considerable difference in the size of the glands of the chicks grown under the various light filters. In general the glands of chicks whose diet contained cod-liver oil are appreciably smaller than those of chicks without the oil but growing under the same glass. This is true except in the chicks of the vitaglass groups. Here it happened that the gland selected for reconstruction from the group in which cod-liver oil was given, at the end of three months exceeded in size that taken from the group given the basic diet. This particular difference in volume does not maintain for all the glands examined of chicks from the vitaglass groups. There appears to be considerable similarity, however, in the size of the glands of chicks under the vitaglass either with or without cod-liver oil in the diet, and thus the lesser wavelengths appear to compensate for the absence of the oil. Among other groups, as the tabulation and figure 2 show, there is a marked difference in the size of the glands of chicks growing under the same glass but fed on the two diets. This difference is far more pronounced under the blue and the amber glass than it is under the ordinary window glass. Actual displacement calculations of the volumes of these reconstructed glands show that under the amber and the blue glass the chicks without the cod-liver oil have parathyroids approximately five times the size of those grown on the basic ration alone.

Histologically these glands are quite consistent. The parathyroids of chicks that were given the oil portion in their diet are usually more nearly

normal in structure, resembling the gland grown under the vitaglass. Evidences of regression in the cell columns are not so pronounced in the chicks of these pens and the function of the gland appears more complete. Occasionally we have noted small cysts in the parathyroids in the chicks under the amber glass on a diet containing cod-liver oil. On the other hand, the marked regression, the breaking down and shrinking of the cell columns, and the development of the greatly enlarged cysts, are characteristic of parathyroids grown under the longer wavelengths, without the oil. It should be stated that here, too, exceptions are noted for in one greatly enlarged gland from a chick under blue glass (fig. 9) no cystic degeneration was encountered and although exactly five times the size of the gland in a vitaglass chick of the same age (fig. 10), the histologic structures are identical. Parathyroid hyperplasia ensues in the absence of the lesser wavelengths of sunlight, but such hyperplasia may be partially obviated by the addition to the diet of a small portion of cod-liver oil.

TABLE 1

*Weight of parathyroid and the body weight of chicks*

| FILTER | AGE | BODY WEIGHT | | WEIGHT OF PARATHYROID | |
|---|---|---|---|---|---|
| | | With cod-liver oil | Without cod-liver oil | With cod-liver oil | Without cod-liver oil |
| | *days* | *kgm.* | *kgm.* | *gm.* | *gm.* |
| Amber glass | 175 | 1.73 | 1.57 | 0.013 | 0.053 |
| Blue grass | 175 | 1.72 | 1.45 | 0.006 | 0.031 |
| Ordinary glass | 175 | 1.75 | 1.74 | 0.009 | 0.0095 |
| Vitaglass | 175 | 1.71 | 1.75 | 0.005 | 0.010 |

On the other hand, parathyroid hyperplasia may also ensue in the absence of the longer wavelengths. In the case of the parathyroid glands of chicks growing under blue glass, marked enlargement was apparent early. Spectrophotometric analyses of the light transmission through blue and ordinary glass show that the blue actually transmits slightly more of the shorter wavelengths than the ordinary glass and yet invariably the parathyroids of chicks grown under blue glass are far more hyperplastic. It would appear then that portions of the entire solar spectrum are essential for the maintenance of the normal activity of these glands. The longer wavelengths alone, transmitted by the amber glass, are inadequate for normal parathyroid activity as are shorter wavelengths and wavelengths of the middle region of the spectrum transmitted by the blue glass. But wavelengths transmitted by both vitaglass and ordinary glass are effective in producing a gland more nearly typical of the normal gland out of doors.

Light, then, appears to bear a definite relationship to the metabolism

of the body associated with the physiology of the parathyroid glands. In the absence of the proper light factors an attempt is made by the organism to compensate for such loss by an increase in the total functional activity of the gland, as shown by its hyperplasia. A superabundance of the secretions of these glands appears to be adequate for the organism devoid of the optimal light factors, for there is no gross evidence, at least, of organic instability. Such compensation appears to be only temporary. In most cases regression is under way in these hyperplastic glands within the first month; it is shown by the breaking down of the cell cords, the increase in the fibroblastic tissue, and the frequent development of the two distinct types of cysts. The causes incident to these phases of regression are not clear. During the earlier weeks of the experiment the parathyroid tissue is apparently normal for each group; but subsequently certain changes arise which may have originated in the body tissue, so that less demand is made on the glands and regression ensues. One may only postulate that at the time regression begins certain cessations, hitherto unrecognized, in the degree of the calcium or phosphorus deposition are getting under way, so that the excess of parathyroid secretion no longer compensates for the loss of the light factor.

## SUMMARY

Studies are reported on the influence of selective solar irradiation on the parathyroid glands of chicks. The chicks were kept within doors behind amber, blue, and ordinary glass and vitaglass which transmit known portions of the sun's spectrum. The percentage of transmission of these various filters is given and discussed. The basic diet used throughout the experiment was the Wisconsin all-mash ration. Half of the chicks under each filter were fed the basic diet alone, while the other half were fed the basic diet and 2 per cent by weight of cod-liver oil. All the chicks of each pen thrived equally well during the earlier months of the experiment; signs of weakness of the legs did not develop until they were five months old.

Enlargement of the parathyroid glands was recognized within the first few weeks in chicks growing under amber and blue glass; this enlargement was due to hyperplasia rather than hypertrophy. The 2 per cent of cod-liver oil added to the basic diet serves to maintain a more nearly normal-sized gland even in the longer wavelengths of sunlight.

Both the longer and the shorter wavelengths of sunlight are essential to the maintenance of normal parathyroid glands.

Regression of the cell columns is manifested early; the cause is undetermined.

Cysts accompany the retrogressive processes that ensue within the gland. These cysts are of two types. One is walled off from the remaining portion

of the gland by definite columnar epithelium while the other is enclosed by a series of concentrically arranged flattened cells.

There was no organic indication either in blood calcium, blood phosphorus or general activity that such parathyroid hyperplasia had arisen.

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# THE CAUSE OF CARDIAC SLOWING ACCOMPANYING POSTURAL APNEA IN THE DUCK

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Richet (1899) while studying the resistance to asphyxia of the duck in diving observed the interesting fact that the heart rate of the submerged ducks fell from 100 or more to 20 or less per minute. He saw in this phenomenon a protective mechanism which minimizes the consumption of oxygen by the myocardium. Richet considered this slowing of the heart a reflex response elicited by the wetting of the skin of the beak and nostrils. The skin reflex of inhibitory nature was supposed to decrease the rate of tissue respiration.

Huxley (1913) discovered an entirely new type of apnea resulting from certain peculiar positions of the duck's head and neck in space. She called it postural apnea. She also discovered that there is a slowing of the heart accompanying the postural apnea. Koppányi and Kleitman (1927) confirmed her results and observed the behavior of the heart during postural apneic pauses of several minutes' duration. They found that the normal heart rate (120 to 200 per minute) fell under the conditions of complete postural apnea to about 40 or 50 beats per minute. On several occasions the heart beat only once or twice or not at all in the course of fifteen seconds. When the head and neck were released from their "apnea position," the heart rate like the respiratory rate was suddenly increased.

Since Huxley assumed that postural elements are involved even in submergence apnea, there was a possibility that Richet's explanation of the slowing of the heart might be incorrect and that the slowing of the heart during postural apnea is due to neck and labyrinthine reflexes upon the cardio-inhibitory center. The question, whether the slowing of the heart is a result of the apnea or whether the two are parts of one complex reflex response is analyzed and answered by the experiments presented in this paper.

EXPERIMENTAL. We used a large number of normal domestic ducks (Anas boscas) of plumage of different color and two muscovy ducks (Cairina moschata). All these ducks showed good postural apnea upon

[1] Expense of this investigation partly met by a grant from the Ella Sachs Plotz Foundation for the Advancement of Scientific Investigation.

stretching or dorsiflecting their neck; in fact, no duck was experimented upon unless it showed satisfactory postural apnea. For our experiments we had the duck tied to an animal board in the supine position. The head and neck of the animal projected beyond the edge of the board so that they could be easily dorsiflexed. Sometimes the animal was immobilized by tying together its legs and its wings, and in that case the animal could freely be moved on the board. All operations such as inserting the tracheal and blood pressure cannulae, locating and ligating various structures in the body, were performed under light ether anesthesia, sometimes reinforced by subcutaneous administration of morphine-urethane mixture. In order to obtain simultaneous records of the heart rate and blood pressure, we took regular blood pressure tracings, inserting a 3-way cannula into the right common carotid artery. Care was taken not to injure the air sacs during this procedure. The base line in all our tracings represents 0 mm. Hg pressure. The respiratory rate was recorded by means of a pneumograph tied around the body of the animal, connected with a tambour. Occasionally the pressure in the cervical air sac was registered by cannulating the air sac by means of a larger venous cannula and connecting it with the recording device. References to any special technique will be made later as required by any particular experiment.

In the first series of experiments we endeavored to determine the effect of postural apnea upon the heart rate and upon the systolic arterial blood pressure. The preparation of the ducks for these experiments was done under ether anesthesia, sometimes reinforced by morphine-urethane mixture and after everything was adjusted the wounds were treated with 2 per cent procaine solution; and in case morphine-urethane mixture was already given, the ether anesthesia was discontinued or only a light nitrous oxide-oxygen anesthesia maintained. The obvious reason for this was that under deep ether anesthesia the postural apnea, as is the case with many other reflexes, cannot be elicited. Postural apneic pauses were produced simply by straightening the neck, the vertex of the head directed downward. They usually were of from one to five minutes' duration. The normal heart rate of the duck as determined by blood pressure tracings varied a great deal (sometimes it was found to be necessary to increase the speed of the drum in order to obtain clear cut records of the heart rate). The average heart rate in ten different ducks was as follows: 120, 132, 144, 156, 162, 186, 192, 252, 278, 312 beats per minute.

The slowing of the heart during postural apnea comes on rapidly yet one can detect a gradual development of the slowing, the maximum of which does not come on until after fifteen seconds. In one case the heart rate dropped from 186 to 18 beats per minute in about 15 seconds. More often the decrease of the heart rate was not as great. We had ducks in which

the rate decreased only from 144 to 48 beats per minute, but we also observed a change from 252 to 12 beats per minute.

We also had the opportunity to observe the behavior of the blood pressure during postural apnea in a large number of ducks and experiments. Here we find two very distinct phenomena. One is the stimulation of cardio-inhibitory center manifesting itself in typical "vagus beats," the other is the stimulation of the vasoconstrictor center tending to produce, of course, blood pressure elevation. These two influences are quite antagonistic and very often the former suppresses the latter, and then we not infrequently saw a marked fall in blood pressure. The normal systolic blood pressure of the duck is about 120 mm. Hg. Describing an experiment in which the vasoconstriction was allowed to develop without being much disturbed by vagus inhibition, we may characterize the sequence of events as follows. After postural apnea has been produced the blood pressure drops almost immediately from 120 to 87 mm. Hg and one can see a marked vagal inhibition of the heart. But after 25 seconds the blood pressure begins to rise and in additional 22.5 seconds it reaches its maximum height, in this case 180 mm. Hg. The rise may be abrupt or gradual. In many cases we saw the blood pressure rising from 120 to 215 and more millimeter Hg (fig. 2). Sometimes the maximal and abrupt blood pressure elevations appear when the apnea is interrupted by an actual respiration or an expiratory effort. In several experiments in which the cardiac inhibition was very powerful, marked and prolonged vasodilatation was observed. Describing such an experiment we may say that the normal blood pressure which in this case was 130 mm. Hg fell from its original level to 50 mm. Hg, and the heart rate decreased from 240 to 36 beats per minute (fig. 1). There are many other parallels to this experiment, in which there was a marked fall in blood pressure and a marked decrease in heart rate. Dipping the duck's head into water we find similar phenomena; for instance, the normal heart rate (156) of a submerged duck fell to 33 beats per minute and its normal systolic blood pressure which was 180 mm. Hg dropped to 88 mm. Hg.

Section of the vagi and more certainly atropinization naturally abolish the vagal inhibitions. In such cases during postural apnea the pressor effect comes on sooner and less gradually for it is not counter-balanced by any inhibiting factor. Not infrequently the section of both vagi causes an enormous rise in blood pressure and an inhibition of the respiratory movements which last for at least ten minutes. After that the respiration may be restored but its rate is very slow and the pauses between the respirations very long. During these respiratory pauses, however, there is no inhibition of the heart. In one muscovy duck dyspnea and death followed the section of the vagi.

The postural apnea is thus characterized by two outstanding circulatory

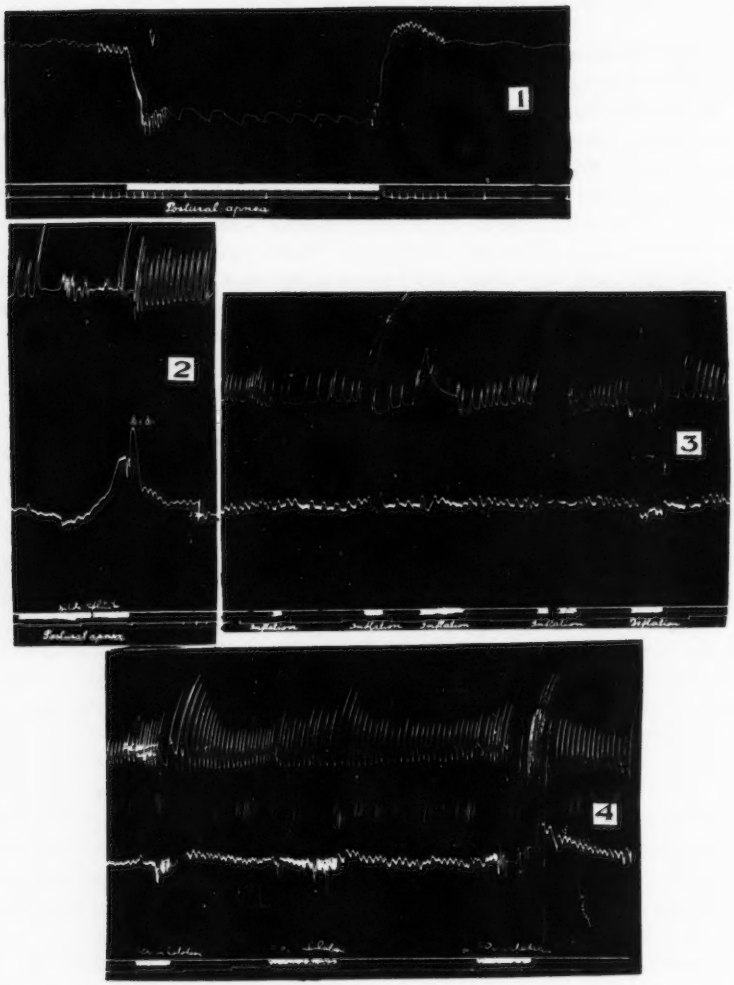


Fig. 1. Blood pressure tracing showing powerful rise in blood pressure during postural apnea.

Fig. 2. Blood pressure tracing showing a marked fall in blood pressure during postural apnea. Note cardiac slowing; slow and fast drum. Time, 5 seconds.

Fig. 3. Simultaneous record of respiration (upper tracing) and blood pressure (lower tracing), showing the effects of inflation and deflation upon respiration and heart rate. Time, 5 seconds.

Fig. 4. Simultaneous record of respiration (upper tracing) and blood pressure (lower tracing), showing the effect of $CO_2$ inhalation on respiration and heart rate. In the first two experiments, especially in the second, the respiration was not seriously interfered with and yet there was a marked slowing of the heart.

phenomena, namely, the cardiac inhibition and the rise of systemic blood pressure. Both are very constant features of the apnea, although as we mentioned, the latter is sometimes suppressed by the prominence of the former. It just so happens that we know that the increased venosity of the blood during asphyxia stimulates both the cardio-inhibitory and the vasoconstrictor centers. So the question suggests itself whether or not the cardiac slowing and blood pressure elevation which we observed during postural apnea are due simply to lack of ventilation and thereby to the increased $CO_2$ tension of the blood. In order to satisfy ourselves on this point it became necessary to perform a great variety of experiments in which apnea was produced by other means than posture. We know that in postural as well as in submergence apnea, cardiac slowing is a constant feature, but according to Huxley, postural reflex may be involved in both. If we produce apnea by factors which do not include postural reflexes, then we still have cardiac inhibition and perhaps elevation of the blood pressure. Then we may safely assume that insufficient ventilation is at least one of the causes in the production of the cardiac slowing and the blood pressure elevation during postural apnea.

*A. Chemical apnea.* It is very difficult to produce apnea vera by the customary means of artificial respiration in the duck. If it appears at all it is of very short duration. Fortunately, we were able to employ a much more efficient method of overventilation. It is a well-known fact that some of the large bones of the duck are hollow, filled with air and connected with the air sacs and lungs. The humeri are connected with the lungs and trachea through the cervical air sacs. Meyer and Meltzer (1916) have shown that it is indeed possible to send a current of air through the humeri and observe the same leaving the body through the tracheal cannula.[2] By this method a very adequate ventilation can be secured. The air current reaches the respiratory surfaces of the lungs of the duck by way of the humeri, cervical air sacs, and saccobronchi. The end pieces of both humeri were removed by a surgical saw, precautions being taken to avoid serious hemorrhages which might obstruct the lumen of the humerus. It is also important to handle those bones very gently and avoid the possibility of a dislocation or disarticulation of the bone. Then both humeri are connected by means of rubber tubings with an insufflation machine in which the pressure under which the insufflation takes place is under control. The animal has been tracheotomized previous to that procedure. The optimum pressure which abolishes the respiratory movements at once lies between 20 and 50 mm. Hg. The administration of atmospheric air or of pure oxygen produces similar results. The chemical nature of this

[2] That the apnea thus produced is not due to distention of the lungs as it was originally assumed by Foa (1909) is proven by the fact that addition of $CO_2$ interrupts the apnea.

apnea is demonstrated by the fact that the addition of small amounts of $CO_2$ to the insufflation gas interrupts the apnea immediately.

If this "per-humeral" insufflation is kept up for a period anywhere from two to ten minutes the respiratory movements do not return at once after discontinuing the insufflation but a very definite apneic pause is produced. Shortly after the appearance of the apnea vera we observed a moderate cardiac slowing which is abolished whenever the first respiratory movements reappear. We must remember that during the apneic pause the arterial blood becomes less and less oxygenated which results in lactic acid formation. This may produce the same effect as high $CO_2$ tension of the blood. The oxygen taken up during the first respirations naturally oxidizes the acid and thus the cardio-inhibitory stimulus may be removed.

*B. Hering-Breuer stimuli.* Hering and Breuer found that if the trachea be closed at the end of an inspiration, the result is a very prolonged expiration, i.e., an expiratory apnea. On the other hand, if the trachea is closed at the end of an expiration, the breathing shows the converse change. These effects are still more exaggerated if one inflates the lungs or allows them to collapse entirely. Hering and Breuer also found that after the section of both vagi the inflation or deflation of the lungs has no immediate influence on the respiratory rhythm. We succeeded in producing apneic pauses in the duck both by inflation and deflation of the lungs. The immediate effect of inflation is the quickening of the heart rate but there is a marked cardiac slowing whenever there is a temporary cessation of respiration following inflation. This result is very easy to interpret, for inflation first furnishes the lungs with plenty of oxygen and the apnea which ensues afterwards produces in turn the usual effects. Deflation (suction) of the lungs causes an immediate cessation of respiration and consequently a primary slowing of the heart, which returns to its normal rate on the recovery of normal respiration (fig. 3).

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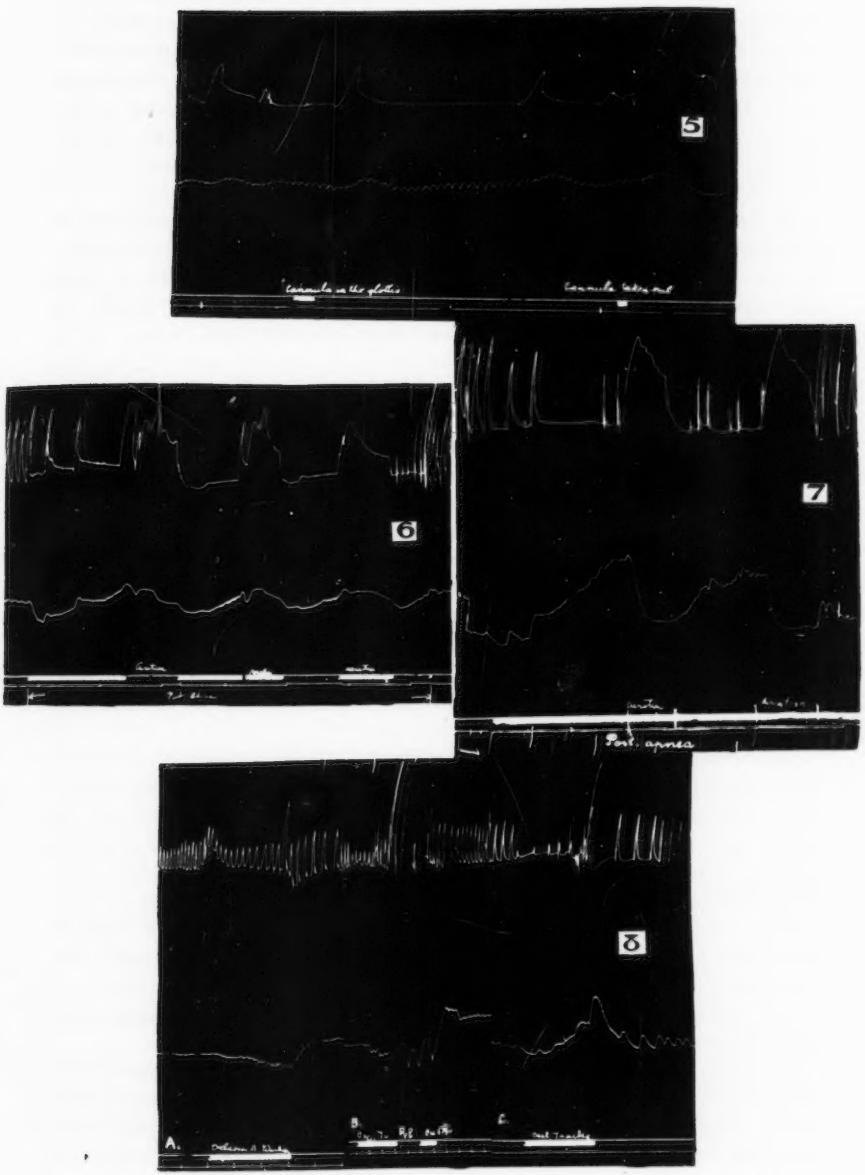
Fig. 5. Simultaneous record of respiration (upper tracing) and blood pressure (lower tracing), showing the effect of stimulation of the glottis on respiration and heart rate. Time, 30 seconds.

Fig. 6. Simultaneous record of respiration (upper tracing) and blood pressure (lower tracing) showing the effect of aeration by means of per-humeral insufflation upon the heart rate during postural apnea.

Fig. 7. Simultaneous record of respiration (upper tracing) and blood pressure (lower tracing) showing the effect of aeration by means of per-humeral insufflation upon the blood pressure during postural apnea.

Fig. 8. Simultaneous record of respiration (upper tracing) and blood pressure (lower tracing). A = effect of the occlusion of the trachea on the heart rate. B = during the occlusion of the trachea the animal's head and neck were placed into the "apnea position" (A. P.). C = occlusion of the trachea accompanied by inhibited respiration during and after the occlusion. Note that the respiratory efforts did not cause any acceleration of the heart rate.


5
6
7
Post. apnea
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*C. Stimulation of the glottis.* We found a hitherto undescribed method to produce apnea in the duck. If one stimulates the glottis of intact or tracheotomized ducks either by placing a cannula into the glottis or pinching with forceps, the duck shows an immediate cessation of respiration which lasts sometimes for one or more minutes after the removal of the stimulus. During these apneic pauses there is a slowing of the heart which is especially marked at the end of the apnea. With the return of the normal respiratory rate the heart immediately speeds up (fig. 5).

*D. Stimulation of the central end of the vagus.* We never observed in ducks an increase in the inspiratory rhythm following the faradic stimulation of the central end of the divided vagus. Whereas very weak currents seemed rather ineffectual, somewhat stronger currents invariably produced cessation of respiration. During the apneic periods there was a corresponding decrease of the heart rate.

*E. Additional observations.* If postural apnea is produced in morphine-urethanized ducks, the respiration is not immediately assumed upon replacing the head and neck in their normal posture. During the apneic pause which follows the postural apnea of morphine-urethanized ducks the blood pressure elevation and cardiac inhibition are still present and disappear only after the restitution of respiration.

Inhalation of ammonia fumes produces a temporary stoppage of respiration which is accompanied by a slow heart.

Inhalation of $CO_2$ (0.4 to 4 per cent) through the trachea or nostrils may or may not produce a slowing or inhibition of the respiration, but always decreases the heart rate (fig. 4). This observation also suggests that the increase of the $CO_2$ in the blood is directly concerned with the production of cardiac inhibition.

These observations show that during apnea, by whatever means produced, slowing of the heart occurs. In most cases, provided the apnea is maintained for a longer time, there is also a rise in blood pressure. These circulatory changes, therefore, are not specific for postural and submergence apnea. Having ascertained this we must examine the problem from a different angle. If the circulatory changes in postural apnea are not specific but are due to insufficient ventilation and accumulation of the $CO_2$ in the blood, we should be able to abolish them by maintaining a sufficient ventilation during reflex postural apnea.

*F. Ventilation experiments during postural apnea.* The sequence of this series of experiments was generally as follows:

Postural apnea was produced and the cardiac and blood pressure effects were observed. Then while the animal was maintained in the postural apneic position air or oxygen was administered and its effect on heart and blood pressure registered. Subsequently the administration of these gases was discontinued and the phenomena caused by this change recorded.

At first we used the customary method of artificial respiration through the trachea. The results obtained by this method were very uniform. The slowing of the heart and the elevated blood pressure (if present) were immediately modified at the beginning of artificial respiration. In spite of the fact that the animal's neck was stretched or dorsiflexed, the heart began to speed up and the blood pressure, if elevated, fell. Then the artificial respiration was discontinued and the heart rate decreased again and vagus beats or blood pressure rise became manifested. Repeated artificial respiration abolished these phenomena again.

The only objection against the experiment lies in the fact that the postural apnea during artificial respiration was no apnea proper but interrupted by periodic distention and collapse of the lungs. The thorax was not at rest. In order to abolish these periodic oscillations and to insure a quiet state of the thorax-abdomen, we returned to the method of per-humeral insufflation. We produced postural apnea and then started the insufflation through the humeri. Now, of course, the postural apnea was not interrupted by periodic movements. The results were exactly the same as in the previous experiments. Per-humeral insufflation with air or oxygen resulted in an immediate quickening of the heart rate. To describe one experiment out of many, the normal heart rate of duck 12 was 144 per minute, 74 during postural apnea, 140 during per-humeral insufflation, and 80 per minute when the insufflation was discontinued (fig. 6). The blood pressure rises are similarly affected by per-humeral insufflation. Aeration caused an immediate drop in blood pressure amounting from 50 to 120 mm. Hg (fig. 7).

It is interesting to note that closure of the nostrils or trachea during per-humeral insufflation produces an almost immediate bradycardia. If $CO_2$ is added to the air or oxygen for purposes of per-humeral insufflation then the respiratory oscillations are not abolished yet there is slowing of the heart. In one case the normal heart rate—312 per minute—fell during $CO_2$ plus air insufflation to 184 beats per minute.

*G. Occlusion of the trachea.* In every instance the occlusion of the trachea was brought about by clamping the rubber tubing which was attached to the tracheal cannula. This procedure, contrary to expectations, never seems to produce exaggerated respiratory efforts. From the very beginning there is a tendency to inhibit the respiratory effort so the picture which follows the occlusion of the trachea in the duck is quite different from that which happens in mammals. During the first minute after occlusion we can usually count not much more than from two to ten respiratory efforts. The slowing of the heart becomes very evident after five to fifteen seconds following occlusion and there is no acceleration of the heart with the respiratory efforts (fig. 8A). However, if we make a single respiratory effort successful by opening the tracheal cannula for a second,

the heart immediately speeds up. The heart rate during occlusion of the trachea was not influenced by straightening the neck ("postural apnea"); this latter procedure neither hastened nor delayed the onset of cardiac slowing (fig. 8B). This series of experiments shows that the lack of respiratory movements itself is not responsible for the cardiac inhibition. The lack of respiratory movements is a factor only insofar as it makes ventilation impossible. Not infrequently it happens that after opening the tracheal cannula the respiration continues to be slow and shallow and it takes some time before the normal respiratory rate is restored. Naturally, during the respiratory pauses which follow the occlusion there is a corresponding slowing of the heart (fig. 8C).

In order to get a better picture of the behavior of the heart during apnea, we took electrocardiographic tracings of the duck before, during, and after postural apnea. These experiments revealed that during apnea there is a tendency to premature ventricular contractions. We were able to observe both the extrasystole and the compensatory pause.

Generally it was found that inspiration quickens the heart and expiration tends to slow it. This phenomenon has already been observed by Huxley.

Discussion. We hope that in this paper certain facts are brought out which throw some light on the problem of cardiac inhibition during postural or any kind of apnea and slow breathing. Different authors have endeavored to give different explanations of the cause of this cardiac slowing. We have mentioned already that Richet believed it to be a skin reflex of inhibitory nature (cardiac slowing during submergence apnea). Lombroso (1913) made a careful study of the causes of cardiac slowing during submergence apnea. He found that submerging the beak of a duck produces apnea and slowing of the heart but when he blew air into the trachea this procedure was followed by almost immediate acceleration of the heart. He considered the possibility that cardiac inhibition might be brought about by the $CO_2$ accumulation due to insufficient pulmonary ventilation. But he considered this factor to be eliminated when he found that occlusion of the trachea did not produce any appreciable slowing of the heart. Lombroso's failure to obtain cardiac inhibition after occlusion of the trachea may be explained by the assumption that the violent respiratory efforts of his animals produced circulation of the air from the filled air sacs through the lungs. Huxley (1913) and Paton (1914) believed the cardiac inhibition which occurs during submergence and postural apnea to be an accompaniment of the apnea, with later asphyxial effects superadded. According to Miss Huxley the gradual decrease in the rate of the heart seen in the majority of the experiments and the rapid change to a quicker beat with the first free respiration indicate that the slowing of the heart is asphyxial in origin; but the comparatively slight fall in rate of the heart where respiration was continued most regularly and the fact

of the slight quickening of the heart with the earlier respirations indicate that another element is at work which tends to regulate the heart rate to the rate of respiration. What this other element is Miss Huxley fails to tell us. She herself proved, however, that not only the maintenance of postural apnea or submergence apnea but also the occlusion of the trachea results in a striking fall in heart rate. Artom (1925) carried out a number of interesting investigations on the effect of submergence upon the respiration and heart rate of diving and non-diving birds. He found that continuous insufflation according to the method of Meyer and Meltzer prevents the cardiac slowing during submergence apnea. When he added 20 per cent $CO_2$ to the air he found that there was an appreciable slowing of the heart. He believed that the accumulation of $CO_2$ is chiefly responsible for the production of the cardiac inhibition during submergence apnea. Koppányi and Kleitman left the question open, although they admitted the possibility that a postural reflex upon the vagus center might be responsible for the slowing of the heart.

Our present experimental data show that the insufficient ventilation and the accumulation of $CO_2$ in the blood is alone responsible for the slowing of the heart, not only in the case of postural and submergence apnea, but in case of any apnea by whatever means it is produced. The increased venosity of the blood stimulates not only the respiratory center but also and much more powerfully, the cardio-inhibitory center in the medulla, thus producing the slowing of the heart in every instance where the pulmonary ventilation is insufficient or absent. The increased $CO_2$ tension of the blood has no direct influence on the heart muscle, for when the vagi are cut or the animal is atropinized, there is no slowing of the heart in spite of apnea or occlusion of the trachea. In fact, we have seen in our experiments that normally the heart rate always tends to keep pace with the rate of respiration and increased or decreased respiratory rate always produces a corresponding increase or decrease in the heart rate. These facts are in close agreement with the findings of Y. Henderson (1908) who has shown that in the dog with open thorax it is possible to regulate the heart rate artificially by increasing or decreasing the artificial respiration. From a normal rate the heart could be slowed down for a few minutes at a time, to rates of only 20 to 30 beats per minute. In dogs of moderate size the heart rate within 2 or 4 minutes could be accelerated from 50 or 60 up to 180 to 200 per minute. It is remarkable, however, that in the duck this regulation of the heart rate by the extent of pulmonary ventilation can be accomplished so much more quickly than in the dog, for instance. Few seconds, instead of few minutes are sufficient to bring about a profound change in the heart rate. According to an hypothesis advanced by Batelli and Stern (1908), the vagus is less excitable in birds than in mammals but the duck is an exception to the rule. According to them, the inhibitory

action of that nerve upon the heart is more marked than in any other animal. This may explain the fact why the heart slows more quickly and markedly in the duck than in other animals. Unfortunately, however, Paton (1913) failed to confirm their observations. He found that vagal inhibition is exercised not only on the auricles but also on the ventricles of the duck, but powerful stimulation is generally required to produce it. It seems to us that no matter whether Batelli and Stern are correct or not the cardio-inhibitory center of the duck is unusually susceptible to changes in $CO_2$ tension of the blood. This center is in a condition of permanent excitability, and according to Epifanio (1912), it can be more easily excited during expiration than during inspiration. Apparently in the duck the threshold of the center for $CO_2$ stimuli is very low.

## SUMMARY

1. There is a slowing of the heart during postural and submergence apnea, reaching its maximum after 15 seconds.

2. The blood pressure may rise during postural apnea, the rise sometimes amounting to more than 100 per cent. This blood pressure elevation is not infrequently suppressed by powerful vagal inhibitions.

3. Section of the vagi or atropinization abolished the vagal inhibitions during postural apnea and permits the early appearance of blood pressure rises.

4. That the cardiac inhibition and blood pressure elevation are due to insufficient ventilation and increased $CO_2$ content of the blood is supported by the following experiments:

a. In the latest stages of the apnea, which follows acapnia, there is a slowing of the heart.

b. The apneae, which follow the Hering-Breuer stimuli, the stimulation of the glottis, the stimulation of the central end of the divided vagus nerve, the inhalation of ammonia fumes, are characterized by corresponding slowing of the heart and by a more or less definite rise in blood pressure.

c. During respiratory pauses, which follow the postural apnea of morphine-urethanized duck and the prolonged occlusion of the trachea, there is a marked slowing of the heart.

d. Occlusion of the trachea itself is followed by gradual decrease in heart rate, which quickens at once when the animal is allowed to respire.

e. Continuous insufflation of the animal during postural apnea prevents the appearance of cardiac inhibition and blood pressure elevation, or when these are already in evidence, it speeds up the heart rate again and lowers the blood pressure almost immediately.

f. Administration of $CO_2$ produces a slowing of the heart.

5. Premature ventricular contractions may frequently be observed during postural apnea.

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# THE EFFECT OF WITHDRAWAL OF WATER ON THE SALIVARY CONDITIONED REFLEX INDUCED BY MORPHINE

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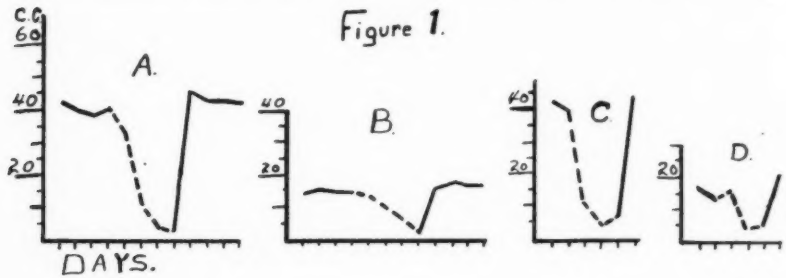
In an earlier paper on a quantitative study of a salivary conditioned reflex (Kleitman and Crisler, 1927) the effect of starvation upon the reflex was reported. In considering the mechanism responsible for the deterioration of the reflex during a period of starvation, Doctor Carlson suggested that the dogs might not drink so much water during this period and the decrease in saliva might then be simply a measure of the lack of water. Kunde (1923) reported that "the desire for water was almost nil" in herself during a 15-day starvation period. Kleitman (1927a) showed that dogs drink only about one-half as much water during starvation as they do normally. The water intake is still further reduced by the amount in the food, so that the actual water intake during starvation is only one-fifth to one-third normal.

Kleitman (1927b) studied changes in the secretion rate of saliva induced by pilocarpine during starvation. He found a decrease during this period. From his data he concludes that "the practical abolition of the morphine salivary conditioned reflex in starvation is probably the result of a physiological deficiency in the central nervous system." The present paper was planned to get at the mechanism of this change, by determining if possible whether the starvation or the water dearth were the important element. In the original paper (Kleitman and Crisler) it was shown that withdrawal of water alone significantly depressed the reflex. Data are now given which confirm those findings, and tend to indicate that the water is relatively more important than the starvation in the abolition of the morphine-salivary conditioned reflex.

METHOD. The present data were secured from five dogs, three of which were used in several consecutive tests. A fistula was made as reported previously (Kleitman and Crisler) and the saliva was measured every five minutes. In the experiments with morphine the test period each day was the thirty minutes after stocking the animal (before the subcutaneous injection of 30 mgm. of morphine sulphate) and the fifteen minutes immediately following the injection. In the experiments with pilocarpine the

dogs were daily injected subcutaneously with 10 mgm. of the nitrate immediately after being stocked and the secretion of saliva measured for one hour.

A typical conditioned salivary reflex was developed in the dogs by means of morphine. When the secretion rate reached a plateau, drinking water was withheld and twice daily the animals were given 200 cc. of 3 per cent sodium chloride solution by stomach tube. This was continued for three,



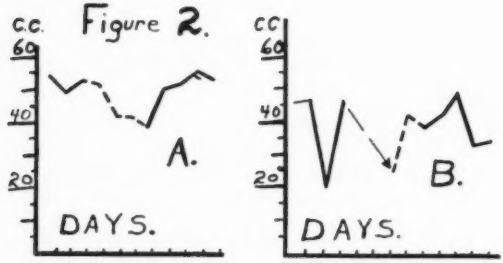




Fig. 1. The solid portion of the curve represents the volume of saliva during liberal water allowance; the dotted portion the volumes during "dehydration." A. Effect of "dehydration," accentuated by 3 per cent sodium chloride, on morphine-conditioned salivation. B. Effect of the same treatment on morphine unconditioned salivation. C. Effect of "dehydration" by simple withdrawal of drinking water on conditioned secretion, and D, on unconditioned secretion.

Fig. 2. The effect of "dehydration," A. Using 3 per cent sodium chloride, and B, withdrawing drinking water, on the secretion of saliva induced by pilocarpine.

four or five days as the case seemed to justify. The dogs were fed and treated the same as in the control experiments in every other way.

After data were secured from the morphine experiments, the conditioned reflex was abolished and the dogs were used in a second series of tests in which pilocarpine was substituted for morphine. Results were obtained, as previously with morphine, under conditions of sodium chloride treatment (10 per cent and 3 per cent) and of simple withholding of water.

Results. Detailed data are presented for three dogs. The sodium

TABLE 1

*Cubic centimeters of saliva secreted conditionally (I) and unconditionally (II) on successive days by salivary fistula dogs, from subcutaneous injections of 30 mgm. of morphine, during periods of normal watering, "dehydration" using 3 per cent sodium chloride solution, and "rehydration"*

| DOG A1 | | DOG A2 | | DOG B1 | | |
|---|---|---|---|---|---|---|
| I | II | I | II | I | II | |
| 42.8 | 15.3 | 42.9 | 17.3 | 48.7 | 20.1 | Period of normal watering |
| 40.1 | 16.1 | 41.9 | 15.8 | 46.6 | 26.0 | |
| 38.4 | 15.7 | 41.1 | 15.0 | 53.3 | 20.6 | |
| 40.1 | 15.1 | 41.4 | 12.9 | 51.0 | 25.9 | |
| | | | | 45.2 | 24.7 | |
| 34.5 | 14.7 | 20.1 | 15.5 | 21.2 | 11.4 | Period of "dehydration" (withdrawing water and giving 400 cc. of 3% sodium chloride by stomach tube daily) |
| 12.7 | 11.9 | 24.1 | 12.3 | 12.4 | ? | |
| 3.8 | 6.9 | 19.9 | 14.8 | 11.0 | 11.2 | |
| 2.7 | 2.7 | 12.5 | 12.6 | | | |
| | | 4.9 | 6.4 | | | |
| 45.5 | 16.8 | 42.5 | 16.0 | 67.4 | 27.0 | Period of "rehydration" |
| 43.8 | 18.1 | 45.3 | 15.4 | 63.7 | 23.6 | |
| 43.1 | 17.5 | 46.6 | 17.7 | 74.4 | 23.5 | |
| 42.9 | 17.3 | 41.1 | 17.1 | 69.2 | 21.3 | |
| | | 41.6 | 17.6 | | | |

TABLE 2

*Cubic centimeters of saliva secreted conditionally (I) and unconditionally (II) on successive days by a salivary fistula dog, from subcutaneous injections of 30 mgm. of morphine, during periods of normal watering, "dehydration" by simple withdrawing of drinking water, and "rehydration" (dog A)*

| I | II | |
|---|---|---|
| 42.9 | 16.5 | Period of normal watering |
| 40.0 | 13.0 | |
| 12.9 | 15.9 | Period of "dehydration" from simple withholding of water |
| 4.3 | 4.1 | |
| 7.4 | 6.1 | |
| 44.8 | 20.3 | Period of "rehydration" |

TABLE 3

*Cubic centimeters of saliva secreted in one hour on successive days by salivary fistula dogs, from subcutaneous injections of 10 mgm. of pilocarpine nitrate during periods of normal watering, "dehydration" by 3 per cent sodium chloride solution, and "rehydration"*

| DOG Br | DOG A | DOG Bl | |
|---|---|---|---|
| 57.3 | 53.9 | 85.3 | Period of normal watering |
| 50.8 | 49.3 | 52.7 | |
| 43.4 | 52.2 | 54.6 | |
| 39.9 | 51.1 | 64.1 | Period of "dehydration" (drinking water |
| 40.8 | 42.4 | 76.5 | withheld and 400 cc. of 3% sodium |
| 29.9 | 41.2 | 59.6 | chloride by stomach tube each day |
| 39.0 | 38.8 | 56.3 | |
| 39.0 | 50.3 | 62.8 | Period of "rehydration" |
| 41.2 | 52.0 | 78.3 | |
| 45.7 | 55.3 | 83.5 | |
| 34.3 | 53.9 | 73.3 | |

TABLE 4

*Cubic centimeters of saliva secreted in one hour on successive days by salivary fistula dogs, from subcutaneous injections of 10 mgm. of pilocarpine nitrate, during periods of normal watering, "dehydration" by withholding water, and "rehydration"*

| DOG Br | DOG A | DOG Bl | |
|---|---|---|---|
| 46.4 | 48.6 | 95.8 | Period of normal watering |
| 48.0 | 48.7 | 101.3 | |
| 21.7 | 48.7 | 89.1 | |
| 46.3 | 56.1 | 94.9 | |

The results of the first two days' "dehydration" are not accurate, and are not included here. The following data is for the third, fourth and fifth days of "dehydration"

| | | | |
|---|---|---|---|
| 25.7 | 41.1 | 71.3 | Period of "dehydration" by simple |
| 42.2 | 33.6 | 34.1 | withholding of water |
| 38.6 | 19.6 | 42.8 | |
| 43.0 | 35.6 | 81.3 | Period of "rehydration" |
| 49.2 | 56.0 | 88.9 | |
| 33.8 | 48.0 | 78.3 | |
| 34.2 | 45.2 | 75.3 | |

chloride treatment was found to abolish the morphine-salivary conditioned reflex more rapidly than does starvation. Three to five days of such treatment leads to almost complete abolition (see fig. 1 A).

The unconditioned secretion of saliva, after the morphine injection, was also decreased by the sodium chloride treatment, but not to the same extent as the conditioned secretion. The nature of this morphine-salivary unconditioned secretion curve resembled the pilocarpine secretion more than the morphine-salivary conditioned curve, figure 1 B.

Withholding of drinking water produced the same effect even in the absence of sodium chloride treatment (fig. 1 C and D). Table 1 shows the

TABLE 5

*Comparison of the effects of "dehydration" on morphine-conditioned and unconditioned salivary secretion, and on pilocarpine salivary secretion, calculated (I) as per cent change from the extremes of "watered" and "dehydrated" secretion volumes, and (II) as per cent change from the average "watered" and "dehydrated" secretion volumes*

| | DOG | EXTREME HIGHEST TOTAL SECRETION | EXTREME LOWEST TOTAL SECRETION | PER CENT DECREASE I | AVERAGE WATERING SECRETION | AVERAGE DEHYDRATION SECRETION | PER CENT DECREASE II |
|---|---|---|---|---|---|---|---|
| Morphine conditioned | Br | 45.5 | 2.7 | 94 | 42.1 | 13.4 | 68 |
| | A1 | 46.6 | 4.9 | 89 | 42.7 | 16.3 | 55 |
| | B1 | 74.4 | 11.0 | 87 | 57.7 | 14.9 | 74 |
| Morphine unconditioned | Br | 18.1 | 2.7 | 85 | 16.5 | 9.1 | 45 |
| | A1 | 17.7 | 6.4 | 63 | 16.1 | 12.3 | 21 |
| | B1 | 27.0 | 11.2 | 59 | 23.6 | 11.3 | 52 |
| Pilocarpine unconditioned | Br | 57.3 | 29.9 | 48 | 44.4 | 37.4 | 16 |
| | A1 | 53.9 | 38.8 | 28 | 52.4 | 43.4 | 17 |
| | B1 | 85.3 | 56.3 | 34 | 70.0 | 64.1 | 8 |

effect of 3 per cent sodium chloride by stomach tube on the morphine-salivary conditioned reflex, and upon the morphine-salivary unconditioned secretion. Table 2 shows the effect of simply withholding water.

The sodium chloride treatment caused a decrease in the average amount of saliva secreted from an injection of pilocarpine, similar to the decrease which Kleitman (1927b) found during starvation (fig. 2). However, there is nothing which compares with the effect on the morphine-salivary conditioned secretion. When water was withheld without the sodium chloride treatment (fig. 2 B) results of the same general type were obtained. Table 3 shows the result of treatment with 3 per cent sodium chloride solution on

the pilocarpine-salivary unconditioned secretion. Table 4 shows a similar effect from withholding drinking water.

In table 5 is summarized the effect of dehydration on three different dogs, *a*, with respect to the extreme variations, under conditions of watering and of water dearth, of salivary secretion conditioned by morphine, and unconditioned by pilocarpine; *b*, with respect to the average variation under the same conditions.

Discussion. Decreasing the water intake of a dog produces a more rapid abolition of the morphine-conditioned reflex than does starvation. The conditioned secretion of saliva may drop from over 40 cc. to less than 3 cc. after four days without normal water intake. The general nature of the curve seems independent of whether or not the condition is accentuated by sodium chloride diuresis. A comparable degree of abolition is not accomplished by eleven days of starvation (Kleitman and Crisler).

During the periods in which dogs receive no water they also do not eat as much as normally. Just as Kleitman (1927a) showed that starving dogs do not drink as much water as normal, so a "thirsting" dog does not eat as much as normal. Whether this indisposition to food is caused by a central (appetite) or a peripheral (hunger) change is being investigated. The effect of water dearth must then function also somewhat indirectly through the "partial starvation" which it effects. However, the decrease in the curve of conditioned secretion caused by the first four days of total starvation is scarcely outside the range of daily variation, so that in these experiments the effect of the partial starvation must be insignificant.

In a series of preliminary tests, not reported, the urine volumes of the animals were followed. A solution of 3 per cent sodium chloride by stomach tube was given to accentuate the water deficiency. In one case the average volume before "dehydration" was 287 cc. for twenty-four hours, during "dehydration" 377 and during "rehydration" 110. In another animal the values respectively were 85, 120 and 70 cc. These increases in urinary volume, coupled with a much reduced water intake, may be assumed to indicate that the animal is in a state of water deficit, or of partial dehydration. The fall in the salivary volumes during such periods of "dehydration," then, must be considered first of all in the light of the possibility that the decreased salivary flow may be simply a reflection of the deficiency of water for saliva elaboration. That an actual deficit of water does exist is indicated by the low level, below normal, at which the urine volume stays even after sodium chloride is no longer given, suggesting a use of the water to restore the tissues. Our first interpretation, then, arguing from the inverse variation of urine and salivary volumes, was that the water was utilized in an effort to get rid of the excess salt and thus was not available for saliva formation. This undoubtedly does play a considerable rôle in the experiment, but that some other factors are pri-

marily involved is shown by the relatively large volumes of saliva secreted after the injection of morphine, and by the pilocarpine experiments during dehydration (table 5).

The morphine unconditioned secretion of saliva is depressed by "dehydration" but not so much as the conditioned secretion. The morphine unconditioned curve resembles the pilocarpine curve more than the morphine-conditioned curve.

That the morphine-unconditioned secretion is depressed more than the pilocarpine is not surprising. The so-called unconditioned volumes collected during the 15 minutes after the injection of morphine are in reality composite volumes made up of unconditioned secretion and of residual conditioned secretion for a while before it is depressed. The greater depression of the unconditioned secretion may then be due to an early "removal" of this conditioned component.

To test the effect of "dehydration" on the peripheral salivary mechanism, pilocarpine was substituted for morphine. Like Kleitman (1927b), we never found a case of conditioned salivary secretion, even after prolonged pilocarpine use. The curves of secretion induced by pilocarpine are very uneven.

The effects of dehydration cannot be explained entirely by the effect on the peripheral mechanism. It must cause some central change of the nature of a depression. That "dehydration" itself is the important element seems evident from elimination. There is little evidence for a pharmacological action on the part of sodium chloride, for the paralleling of effects in cases in which it was and was not used rules out "salt poisoning."

In contra-distinction to dogs on liberal water allowance, the "dehydrated" animals usually ate within 15 minutes after the test, they never vomited, and showed much less depression from the morphine. These facts lend additional support to our interpretation that dehydration depresses at least some of the central nervous processes.

Two inferences suggest themselves. That the effect of starvation is mainly indirect through its "dehydrating" action, and that the conditioned and unconditioned stimuli involve, in part, different central mechanisms. The differential decrease in the conditioned and unconditioned secretion of saliva may mean that the unconditioned stimulus, morphine, transported by a humoral route, stimulates the "center of nausea" directly, while the conditioned stimulus (or stimuli) acts upon this center indirectly.

## CONCLUSIONS

1. The conditioned secretion of saliva is practically abolished by three to five days of "dehydration."

2. Unconditioned salivary secretion (pilocarpine and morphine) is reduced by "dehydration," but much less than the conditioned secretion.

3. The peripheral mechanism involved in conditioned salivary secretion is little affected by this "dehydration," but certain central processes are depressed.

4. Dogs deprived of water eat less than normally.

5. Since the depression of the morphine-salivary conditioned reflex during starvation with water ad libitum is less than during total absence of water with food ad libitum, the water element appears more important than starvation in the depression.

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# CONDUCTION IN DEPRESSED CARDIAC MUSCLE AND ITS RELATION TO THE THEORY OF PARTIAL HEART BLOCK[1]

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In correlating the findings in pressure block of the A-V bundle in dogs with those obtained with cold-blooded heart strips, Erlanger (1906) was led to propound an hypothesis to account for the facts of partial block. Briefly stated, this view took into account the interaction of two factors, namely, the normal irritability changes in the ventricle, and the strength of the impulse coming from the auricle. If, in the course of recovery of irritability in the ventricle following preceding systole, the strength of the auricular impulse is sufficiently great to reach the threshold of irritability of the ventricle, a contraction follows. If, however, in its course through the injured area, the strength of the impulse is diminished so that by the time it arrives in the ventricle it has become subminimal, the ventricle fails to respond. The hypothesis, it will be noted, presupposes differences in the reactivity of the muscle along the path taken by the impulse.

Lewis and Master (1926), in an endeavor to clarify further the factors involved in the production of halved rhythm and block in general at the A-V junction of the mammalian heart, have induced halved rhythm by vagal stimulation, quinidine poisoning, asphyxia, etc., and during its continuance have shifted the position in time at which an alternate ineffective stimulus is sent into the auricle. In correlating the duration of the P-R interval of the succeeding effective stimulus with the position in time at which the intermediate ineffective stimulus is placed they believe they have found between the two effective stimuli a very brief period, termed by them the "phase of interference" ("lasting when demonstrable about 0.02 to 0.09 of a second"), during which an interposed stimulus, though ineffective, alters the conduction rate of the succeeding impulse. Upon the basis of this they argue that if the idea put forward by Erlanger, that the length of the P-R interval is influenced by the strength of the impulse,

[1] The experimental data in this paper are taken from the dissertation presented by Francis O. Schmitt in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Washington University, 1927.

holds, then in their experiments as the interval between the ineffective auricular contraction and the following effective one decreases, the length of the P-R interval of the latter should increase *gradually* over a *considerable* phase of the cycle. In their experiments on the contrary, they say, "the increase is not gradual, it is abrupt, it is not really progressive, and the phase of interference is brief . . . .". Now, as a matter of fact, when

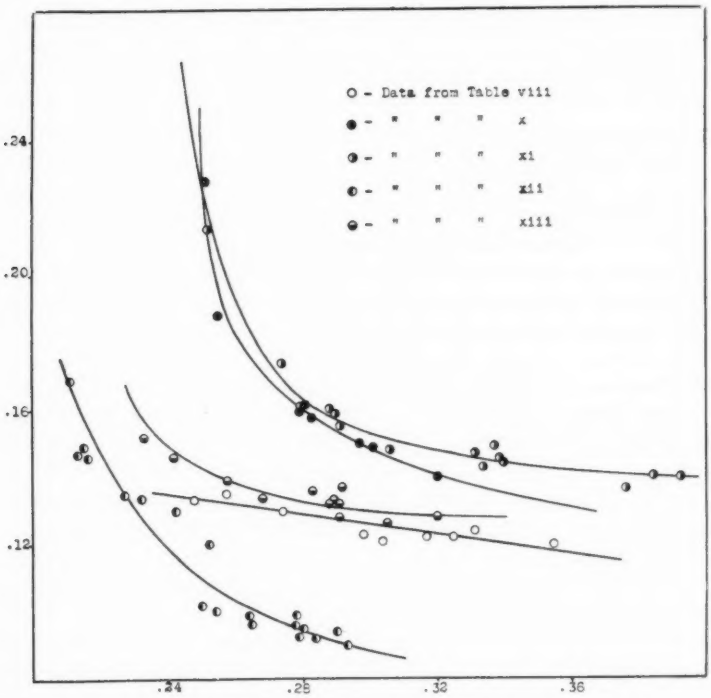


Fig. 1. Data of Lewis and Master plotted to show the gradual and progressive shortening of $P^2_s$-$R^2$ as $P^2$ lags behind $P^2$. Ordinates are $P^2_s$-$R^2$ periods, abscissae, $P^2_s$-$P^2_s$ periods.

their data are plotted in the usual way, instead of in the diagrammatic manner used by them, their "$P^3_s$–$R^{3}$" values as ordinates, their "$P^2_s$–$P^{3}_s$" values as abscissae, it becomes clear (see fig. 1) that within the limit of error the shortening actually is gradual and lasts throughout the periods they have tested, though it proceeds, not unexpectedly, along a curve that is concave upwards. Ashman (1925), independently, has studied the same phenomenon in the turtle's heart. He shows, likewise, that the

effect is continuous; and again, that the later the blocked impulse falls the longer is the subsequent A-V pause. Since, then, the effect is gradual Lewis and Master's experiments do not supply the "direct evidence of a destructive kind" to the view that the strength of the impulse plays a rôle in the phenomenon of block.

The explanation that Lewis and Master offer of their so-called phase of interference is that during that very brief period the impulse decrements through the conducting tissue and they "suspect that a similar conclusion may apply to 2:1 block produced . . . . in other ways." The fact that the phase of interference is not so limited in duration as Lewis and Master suppose does not, it should be pointed out, militate against decremental conduction as a possible factor in heart block.

In 1926 Drury, through studies of conduction in compressed and cooled mammalian auricular muscle, was led to conclude that conduction is decremental in injured auricular muscle. His evidence consists of the observation that the conduction rate between three equidistant electrodes under a clamp falls off more rapidly from the second to the third electrode than from the first to the second. This held regardless of the direction of conduction through the block. He found, furthermore, that with the auricle under a compressing pressure that was producing a 2:1 block while the auricle was being driven by stimulation outside the clamp, stimulation under the clamp resulted in a return to the 1:1 rhythm. This, together with other evidence which Drury submits, proves that an impulse may die out in a depressed region and so cause dropped beats.

Under the circumstances it seemed desirable to obtain additional data on the character of conduction in injured heart muscle and, in the event of substantiation of the evidence in favor of decremental conduction, to ascertain whether varying the strength of an impulse affects its propagation through the injured region.

METHODS. To ascertain whether a heart strip conducts decrementally it is necessary to know the moment of arrival of the impulse at three places, at the least, along the course of the injured tissue, as well as at some point in the normal muscle on either side of the injured region. It was desirable, furthermore, that the injury employed be a reversible one, that it be roughly standardizable, and that it come on fairly rapidly lest fatigue effects intervene and obscure the picture. In the search for such a method several ways of impairing conduction were tried, which, although they proved inadequate for the present purpose, furnished valuable data which will be presented in another place and may therefore be mentioned in this connection.

Pressure impairment was the first method tried. The pressure was exerted by a thin sheet of rubber dam pierced by a hole through which the muscle passed. By means of a suitable device the pressure exerted upon

the muscle could be varied by varying the tension on the edges of the sheet of rubber dam. Although all grades of depression, even to complete block, could be produced in this way, the depression was neither sufficiently steady nor controllable. The second method tried was that of electrical polarization. By the use of suitable non-polarizable electrodes, the muscle strip could be subjected to longitudinal bi- or tripolarization or to transverse polarization. Although it was found that the conduction of the impulse could be blocked by these means, even completely, the results were by no means uniform or reproducible. Furthermore, the current usually stimulated the muscle, often to the point of rhythmicity, before blocking occurred, thus frustrating accurate control of conduction intervals. The third method tried was depression of conduction by cold. By means of a circulation apparatus connected to a cooling unit the medial portion of the muscle was cooled to any temperature desired while the two end segments were held at room temperature. This method proved satisfactory in most respects but it was found that the depression of conduction was a straight line function of the temperature only if the temperature change was rapid. When a segment was left at a constant low temperature for some time, the blocking effects rapidly diminished and an habituation to the low temperature developed.

The method finally adopted as the most satisfactory way of depressing conduction was to increase the KCl content of the Ringer solution bathing the medial portion of the strip. A normal Ringer solution was made according to the Bayliss formula containing NaCl–0.65 gram, KCl–0.014 gram, $CaCl_2$–0.012 gram, $NaHCO_3$–0.02 gram, $H_2O$ to 100 cc. When the KCl content of this solution was increased to 0.07 per cent or 0.084 per cent, very marked, yet reproducible and reversible hindrance of conduction resulted as will be described below.

The method of following the conduction of the wave of contraction is shown in figure 2. In the present experiments the muscle was contained in five chambers, namely, two end chambers, *1* and *5*, composed of glass and the medial chambers, *2*, *3* and *4*, composed of hard rubber. Thickening of the muscle in each chamber upon contraction imparts motion to a light counterpoised heart lever through the medium of a very light tubular aluminum connecting rod which rests, by means of a small hard rubber treadle, on the muscle, to which it is held by a projecting, fine platinum pin. The connecting rod, $8\frac{1}{2}$ inches in length, weighed but 0.6 gram, and the muscle was relieved of most of this weight by means of a very light elastic band which could be drawn to any desired tension. The time was recorded in fifths or fiftieths of a second depending upon the speed of the drum. In order to ascertain the magnitude of any inertia differences in the several recording levers, a heavy rubber band was substituted for the muscle and records were made of the displacement of the rubber in response

to a touch at any point, which altered the tension in all parts of the rubber band at practically the same instant. It was found that the four treadles

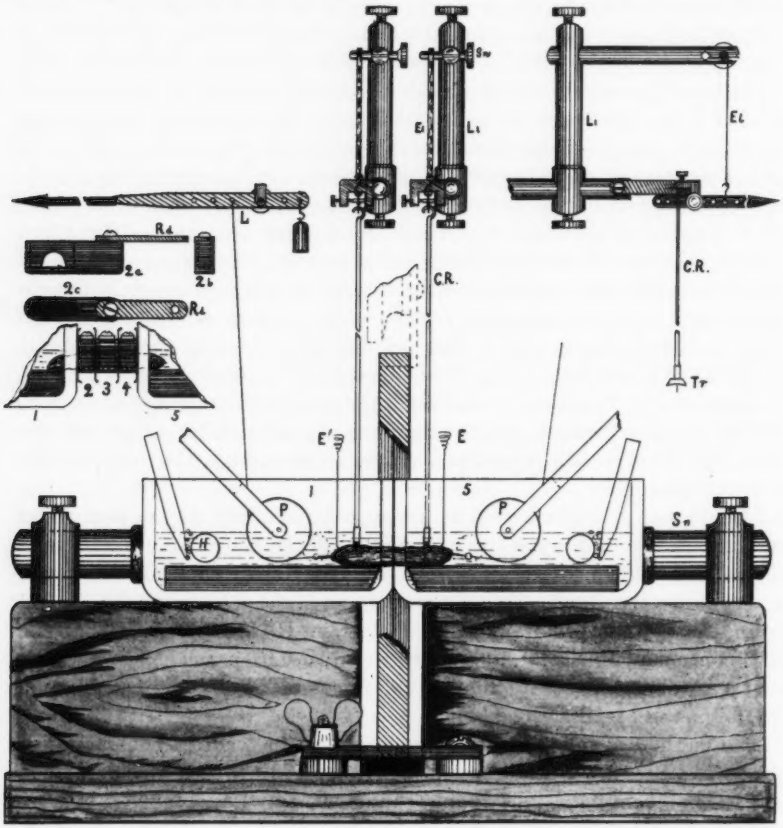


Fig. 2. The chambers, mounted on the wooden base, can be tightly abutted against each other by means of the adjustable snubbers, *Sn*. *2 a*, *b* and *c*, are the side, front and top views, respectively, of one of the intermediate hard rubber chambers. When the muscle is suspended in the five chambers the appearance from the side is as shown (1, 2, 3, 4, 5). *E* and *E'*—electrode pairs, *P*—pulley, *L*—weighted lever stretching muscle along base, *C.R.*—connecting rod, *Tr*—hard-rubber treadle, *Sw*—windlass for adjusting tension of elastic band, *El*, relieving the muscle of most of the weight of the connecting rod and lever.

tested got under way within 5 to 8 sigma of each other regardless of the point of application of the tension. These differences were so small relatively that it was unnecessary to take them into account. Metal parts

dipping into the solution were laquered to minimize oligodynamic effects. The electrodes were of silver or copper. They were connected through a rotary interrupter to a Porter inductorium so that make shocks could be thrown into either end of the muscle at any rate desired.

The preparation consisted of a strip cut parallel to the border of the ventricle of the turtle, *Pseudemys concinna*, the end from the right side of the heart being usually placed in chamber *1*. The strip was held stretched longitudinally by a weighted lever connected to one end of the preparation by a thread passing over a partially submerged hard rubber pulley, the diameter of which was large so that the metal parts could be kept out of the solution. Intermingling of the fluids of the several chambers was prevented by interposing between them thin rubber curtains, through which the muscle passed by means of small, close, but not too tightly fitting holes made by a hot wire. In mounting the preparation the heart strip was drawn through the temporarily stretched hole of a rubber curtain, then through the holes in a hard rubber chamber, through another similar curtain on the other side, and so on. Vaseline served both to hold the curtains to the chambers and to prevent leakage of fluid. Each chamber thus became a water-tight compartment.

To ascertain whether conduction is decremental it is necessary to know the time of arrival of the impulse at not less than three points along the course of the injured tissue. To accomplish this, three hard rubber chambers, *2*, *3* and *4*, of exactly the same dimensions, mounted upon a single brass rod which could be rigidly held in place by a clamp, were interposed between chambers *1* and *5*. The muscle is first threaded through chamber *3* in the manner just described. Chambers *2* and *4* are then swung into line with chamber *3* and the muscle drawn through the holes in these and through the intervening curtains. The three chambers are then bound together by means of small pins protruding from one chamber and fitting into corresponding holes in the adjoining chambers. Finally the ends of the muscle are drawn, one each, into chambers *1* and *5*. In order to obtain sufficiently long muscles, very large turtles were used, from which it was possible to obtain strips often exceeding 6 cm. in length. The four rubber curtains placed on the muscle between the five chambers were sufficiently tight to prevent any tug along the muscle from one treadle to the next. This was an important precaution to observe as the distance between the centers of the three central chambers was but 8 mm. As a result of the local compression exerted by the curtains at four points, and of several other contributing factors, certain phenomena at times made their appearance which will be discussed in a later communication. Fibrillary contractions occasionally developed in the longer strips. It was found that bathing the muscle in Ringer solution containing about 0.25 per cent quinidine sulphate caused the fibrillations to cease. Then, upon

restoring the muscle to fresh Ringer solution the strip became available for the experiment as planned. Figure 3 is typical of the records written by the five levers under the conditions described above. Using a tuning fork vibrating fifty times per second it was usually possible to read the point of rise accurately to 0.01 second.

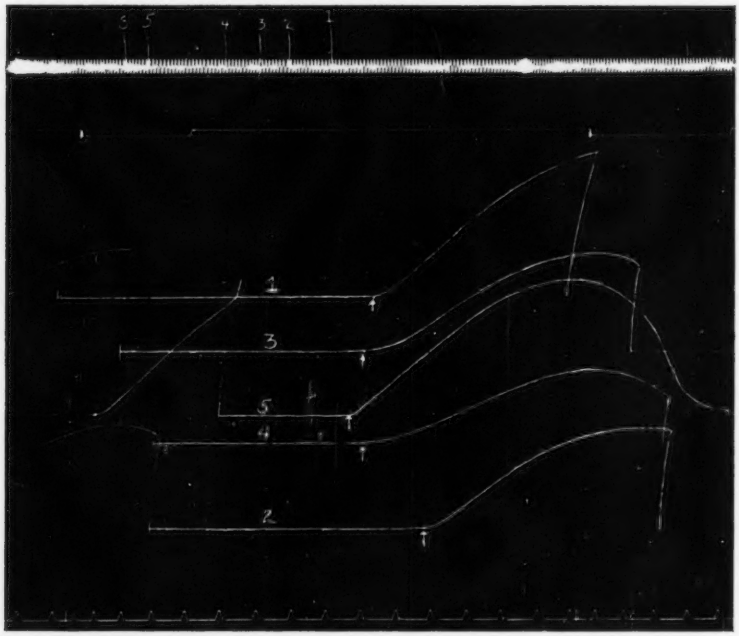


Fig. 3. A record taken from five points along a ventricular strip to show the progress of the contraction wave along it. The numbers refer to the segments of the muscle in the corresponding chambers and to the recording treadles also. The impulse is travelling from *5* to *1*. The top line gives the time in fiftieths of a second; on it are laid off the moment that *5* was stimulated (*S*), and the beginnings of the contraction in the segments *5* to *1*. The moment of stimulation is indicated by a rise in the second line. The lowest line gives the time in fifths of seconds. Reduced one-third.

The steps in the experiments now under consideration were as follows. During the course of a series of contractions started by rhythmical stimulation through the electrodes in chamber *1* or *5*, usually at the rate of about five per minute, which produced a steady state of activity, without disturbing the rhythm six contractions were recorded in succession, namely, two from impulses coursing through the strip in the *1–5* direction, two in the

*5–1* direction, and again a pair in the *1–5* direction. The comparable conduction intervals thus obtained were averaged. The two trials before and the two after the reversal of direction, i.e., the four in the *1–5* direction, showed surprisingly close agreement for each interval; they rarely differed by more than 0.02 second. This difference, amounting to ± 2 per cent of the whole interval, is so small as to exclude as a factor in the data of the present experiments the phenomenon termed by Skramlik "Bahnung der Erregung" in heart muscle. At the same time, the narrowness of the range of the variations testifies to the accuracy of the method. After having made about six to eight sets of determinations in Ringer solution over a period of about thirty minutes, the solution in chambers *2*, *3* and *4* was changed to Ringer solution containing 0.07 per cent to 0.084 per cent KCl, the Ringer solution in chambers *1* and *5* being frequently renewed during the experiment. During 40 to 60 minutes under these conditions, some six to ten determinations were carried out in the manner described above until it became plainly apparent simply by inspection of the records that the conduction rate was decreasing rapidly. At this point, Ringer solution containing 0.07 per cent KCl was placed in chamber *1*. After some four to six determinations were made under these conditions, and when a still further increase in the conduction interval was apparent, normal Ringer solution was again restored to chamber *1*. Two or three determinations were made under these circumstances and then normal Ringer solution was restored to all chambers. It was necessary to gauge as nearly as possible the viability of the muscle during the course of these operations and to vary the length of exposure to the potassium accordingly, lest the effect become no longer reversible.

If the addition of the KCl produces a gradient of conduction, the result in an experiment of this kind should be an increase of the *3–4* interval that is more rapid than that of the *2–3* interval when the conduction is in the *1–5* direction; and, when conduction is in the *5–1* direction, an increase of the *3–2* interval that is greater than that of the *4–3* interval. The treadles were placed crossing the strip as nearly as possible transversely in the middle plane of the chambers, but lest this was not accomplished accurately, comparison is made, not of the absolute conduction intervals, but of their relation, one to the other, as they changed under the treatment to which the muscle was exposed. The data are most easily comprehended when plotted in the manner shown in figure 4. The conduction time from *2* to *3* is divided by that from *3* to *4*. This quotient should be unaffected when KCl is added to the middle chambers if the conduction rate in these chambers falls to its minimum level immediately upon the entrance of the wave into the first chamber and if the rate then remains constant throughout the injured area; but if a gradient of conduction rate is present, this quotient should behave in the manner indicated above. The quotient seldom

remained perfectly constant even though the muscle was in a steady state. Nevertheless, variations due to experimental conditions were usually quite definite.

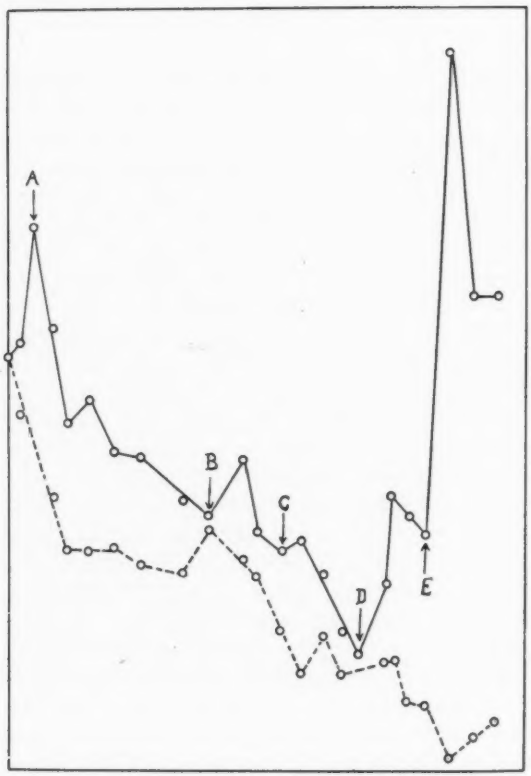


Fig. 4. Illustrating decrement of conduction rate through the depressed region. Ordinates are conduction quotients, abscissae, time in minutes during the experiment. Solid line represents the *2–3/3–4* quotient, broken line the *4–3/3–2* quotient. The sequence of events was as follows: A, Ringer solution containing 0.084 per cent KCl in chambers *2*, *3*, and *4;* B, Ringer solution containing 0.098 per cent KCl in chambers *2*, *3*, and *4;* C, Ringer solution containing 0.084 per cent KCl in chamber *1*, D, Ringer solution in chamber *1;* E, Ringer solution in all chambers.

In five experiments the shape of the curve of the conduction quotient was such as to indicate beyond peradventure that the conduction rate through the depressed region decreased decrementally from one chamber to the next. The proof of decremental conduction is furnished by the fact that

this relation exists irrespective of the direction of conduction (see fig. 4). Two experiments showed a much lower gradient in one direction than in the other. This, however, is not surprising; it is consistent with other irreciprocal relations found to obtain in such preparations, as will be brought out in a later communication. It would appear, consequently, that we have been successful in demonstrating that the conduction rate, and presumably, therefore, the impulse strength, falls off along a gradient when the medial portion of a strip of ventricular muscle is treated with an excess of potassium. In some instances when KCl was added the quotient indicated a conduction increment for a few minutes before the usual decrement. This can only mean that at the time the determinations were made, the strip was already conducting decrementally, and that the addition of the KCl momentarily decreased the conduction gradient. Whether or not the original conduction was one of decrement it is impossible to say, for to have known that it would have been necessary to have had data on the absolute conduction rate through the length of the strip. A response of the same kind, but opposite in sign, is often observed upon restoring the muscle to normal Ringer solution after it has been under the influence of an excess of potassium.

Having demonstrated the presence of decremental conduction, the next step was to ascertain whether the gradient of the decrement can be affected by varying the strength of the impulse passing into the region of the decrement. Our experiments clearly show that it can. Figure 4 exhibits the effect of treating the muscle in chamber *1* with KCl at a time when there is already a steep gradient of conduction rate in the middle chambers. The KCl was added to chamber *1* at *C* and presumably decreased the strength of the impulse generated in chamber *1*, after increasing it initially, due to its preliminary stimulating action. The conduction quotient in the *1–5* direction decreases along a steep gradient, not, however, the quotient in the reverse direction; instead, the latter being unaffected by the changes in chamber *1*, soon comes to equilibrium. This step in the experiment further substantiates the existence of decremental conduction: for, weakening the initial stimulus markedly increases the grade of the decrement through the depressed region. When the initial impulse is restored to its normal value by returning Ringer solution to chamber *1*, the quotient in the *1–5* direction immediately returns to, indeed, temporarily passes even beyond the value it had at *C*, i.e., while the muscle in chamber *1* was still in normal Ringer solution. Meanwhile, the quotient in the *5–1* direction is unaffected, or, if anything, suffers further decrease due, perhaps to a fortuitous decline in the strength of the impulse arising in chamber *5*. Upon restoring the muscle in the middle chambers to normal Ringer solution, the conduction quotient in the *1–5* direction immediately rises above the highest value at the beginning of the experi-

ment, but soon returns to the initial level. The quotient in the *5–1* direction is to some extent similarly affected; its failure to exhibit the same quantitative changes as the *1–5* quotient possibly is to be attributed to greater deterioration of the segment of the strip in chamber *5* than of the segment in chamber *1*.

The results just described are open to but one interpretation, namely, that the steepness of an existing decremental gradient is increased by reducing the strength of the impulse entering it. It follows, furthermore, that, given a long enough or a severe enough stretch of decrement, an impulse may run to extinction and also that the distance traversed before extinction will vary with the initial strength of the impulse.

THEORETICAL CONSIDERATIONS ON HEART BLOCK. In considering the possible rôle of decremental conduction in the phenomenon of partial heart block it must first be ascertained whether it can account by itself for the characteristic aliquot relation of ventricular to auricular frequency. In 2:1 block, for example, it would be necessary to assume an alternation in the effectiveness of the successive impulses. We have found it impossible, however, to conceive of anything resulting from decrement but a continuously decreasing degree of effectiveness, or, at the most, a steady state. And if, for purposes of argument, it be assumed that under a given set of conditions an impulse in traversing subnormal tissue decrements to extinction and therefore fails to stimulate the ventricles, there is no reason why the next impulse should decrement any less; the block under such circumstances must be complete. Evidently a periodic alternation in reactivity beyond the region of decrement is needed to produce the alternation characteristic of partial heart block.

The present experiments, indicating that the conduction rate falls off decrementally through injured tissue, justify the assumption of a parallel decrement in the strength of the associated impulses, for there is no satisfactory reason for separating the several aspects of reactivity of heart muscle. Indeed, their inseparability is supported by the present experiments which show that if the strength of the impulse made to pass through a depressed region is decreased the steepness of the gradient of conduction rate increases. Evidence from many sources supports the same conclusion. According to Schellong (1925), for instance, "*verringerte Intensität der Erregung und verlangsamtes Fortschreiten sind untrennbar verbunden. Auch die Geschwindigkeit des Erregungsfortschreitens ist somit auf den Grad der Erregbarkeit gebunden.*" Since, however, neither the nature of the impulse nor the method of its propagation are understood it might seem presumptuous to speak of strength, since, except possibly in the ability of an impulse to pass an obstruction, there is no way known of measuring impulse strength. There is, however, one view, that popularized by Lillie (1923) on the basis of which it is possible to obtain an objective measure of impulse

strength. According to this view the propagation of the excited state in tissues is due to the effect upon the tissues adjacent to the excited region of currents set up by the potential that develops in the active region. If the resistance of the tissue remain constant the effective spread of current from the active region will depend upon the potential difference developed,

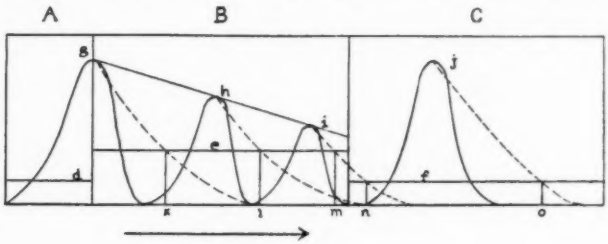


Fig. 5a

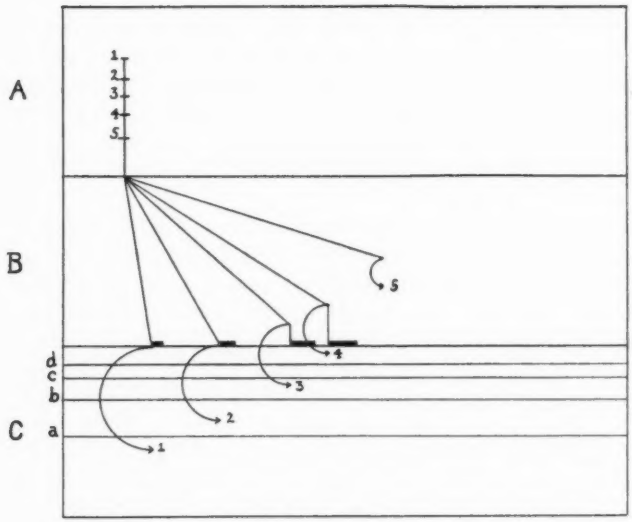


Fig. 5b

upon the rate of rise of the potential, and upon the irritability of the tissue. In other words, the strength of the impulse would vary with the amplitude and the rate of rise of the action potential.

If we accept stimulation by the action potential as a working hypothesis a diagram can be constructed indicating how an impulse may propagate itself through decrementing and blocking stretches of heart

muscle. In figure 5*a*, the action potential wave is shown as it progresses from a normal region, *A*, through a depressed region, *B*, into normal tissue, *C*. The potentials which develop with action are indicated by the solid line waves, the spread of potential into the tissue ahead of the waves by the dotted lines.[2] The irritability of the tissues, that is, the strength of the threshold stimulus, is indicated by the horizontal lines, *d*, *e* and *f*. The tissue will be stimulated by the spreading currents wherever the dotted curves lie above the irritability lines, excepting where the tissues are refractory, as they will be under the fronts, at the very least, of the potential waves. The wave *g*, passing from normal tissue, *A*, into decrementing tissue, *B*, will stimulate as far ahead as *k*. In the decrementing tissue, wave *h* stimulates out to *l*, but wave *i* cannot stimulate ahead of itself in *B*, since the line *m* falls within the refractory phase of *i*. But the potential spread from *i* lies above *f* in the normal tissue, *C*, and stimulates as far out as *n*. The normal tissue, responding to this in accordance with the "all-or-none" law, develops an action potential, such as *j*, and the impulse is then propagated through it in a perfectly normal manner despite the inability of *i* to stimulate *B*. To advance an hypothesis requiring the action potential to stimulate through an unresponding zone of heart tissue may seem to be rather radical, not, however, to anyone who has witnessed stimulation of the phrenic nerve by the action potential of the heart reaching through both the very thick sheath of the former and the epicardial covering of the latter.

Figure 5*b* is a diagram which visualizes all of the above mentioned factors assumed to play a rôle in the transmission of impulses through a region of block. The vertical line in segment *A* represents the strength of the impulse in normal tissue. The impulse is conducted across the region of block, *B*, along the decremental paths shown, the degree of decrement and the time lost in transmission being greater the greater the displacement of the lines to the right. At the *B-C* junction, the junction of decrementing with normal tissue, the factors referred to in figure 5*a* come into play: the bioelectric current, due to the greater fraction of the ascending phase of the action potential required to stimulate, can activate *C* only after a delay of a magnitude indicated diagrammatically by the length of the horizontal oblongs; while the relative stimulating ranges of the bioelectric currents are represented by the diameters of the semicircular arrows. Changes in the threshold of irritability in *C* with the lapse of time following a contraction are indicated by the levels *a* to *d*, *a* representing the lowest level of irritability immediately after the disappearance of absolute refractoriness, and *d*, the highest irritability attained under ordinary circum-

[2] No attempt is made here to correlate irritability and rate of conduction with potential-time or potential-distance relations.

stances. If the curved arrow reaches the threshold level of irritability of segment *C* a contraction will result; if it does not, the impulse is blocked.

Impulse *1*, it can be seen, will stimulate *C* at any time after the disappearance of absolute refractoriness; but impulses *2* and *3* can stimulate only provided some time has elapsed following systole, and impulses *4* and *5* will not stimulate *C* at all. If, at normal heart rates, the irritability of *C* had time toreach level *a*, at half the normal rate, level *b*, and at one-third and one-fourth the normal rates, levels *c* and *d* respectively, impulses of the strength of *1* would produce a 1:1 rhythm, of *2* a 2:1 rhythm, and of *3* a 3:1 rhythm. Impulses of the strength of *4* would stimulate only if, for some reason, the irritability of *C* became abnormally high. The diagram indicates further that though the excited states *3* and *4* fail to traverse *B* completely, nevertheless, under the circumstances just mentioned, they could, through potential spread, stimulate *C*. These examples of the way in which the scheme works should suffice to indicate the methods of working out through it other combinations of impulse strength in *A*, decrement in *B* and irritability of *C* that might lead to the appearance of block phenomena.

At the time the hypothesis of heart block which utilizes variations in stimulus strength and in irritability of the ventricles was worked out (Erlanger, 1906) our knowledge concerning the anatomy and physiology of the conducting system was in a far less satisfactory state than at present. The normal blocking point was then supposed to be the junction of the conducting system with the ventricular muscle. Since then it has been learned that the main delay in the transmission of the impulse through the mammalian heart develops above the A-V bundle, Hering (1910) and Erlanger (1912) both assigning it to the A-V node. Upon the basis of the view elaborated by Engelmann (1895), which the results of the present investigation fail to clearly support, that impulses are transmitted less readily from less reactive to more reactive tissue, the main delay would be at the nodal-funicular junction, for, of the three junctions between the four tissues of different conduction characteristics, namely, auricular, nodal, funicular, and ventricular, it is at the nodal-funicular alone that the impulse passes from slowly to rapidly conducting tissue (Lewis, 1921). Accordingly, the analogously reacting parts in strips as they have been prepared in the present experiments and in the mammalian heart would be segment *1* and the atrium, segments *2*, *3*, and *4* and the A-V node, and segment *5* and the Purkinje-ventricular system. Whether in addition, the nodal tissue normally conducts decrementally or only when its function is depressed by too rapid stimulation, by vagus stimulation, by asphyxia, by quinidine, or by disease, for example, are problems that remain to be solved.

SUMMARY

1. Methods are described by means of which it is possible to satisfactorily depress the conduction rate in a stretch of a heart muscle strip lying between two normal portions and to record the moment of arrival of the contraction at five points along the strip.

2. When the middle stretch is depressed by increasing locally the KCl content of the Ringer solution bathing it five- to sixfold the conduction rate through it falls off along a gradient; since this gradient obtains irrespective of the direction in which the impulse traverses the depressed region, the conduction must be decremental. Weakening the impulse entering the region of decrement steepens the grade of the decrement.

3. Based upon decremental conduction and upon the theory of conduction of excitation which attributes impulse propagation to self-stimulation by the action potential of the local process, a modification of the Erlanger hypothesis is developed which seems to fit satisfactorily the facts of partial heart block.

The author desires to take this opportunity of acknowledging his indebtedness to Prof. Joseph Erlanger for constant aid and advice throughout the prosecution of this investigation and for much valuable assistance in the development of its theoretical aspects.

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*Ann Arbor, Michigan, April 12, 13, 14, 1928*

*The excitability, conductivity and contractility of the turtle heart as influenced by activity.* RICHARD ASHMAN and ROBERTA HAFKESBRING.

The staircase in the turtle heart and the supernormal recovery curve are well-known phenomena, believed to be associated. They have been explained as due to an accumulation of acid metabolites in the muscle, although Adrian,[1] who has called attention to the close parallel between the course of the recovery of excitability and contractility, has rejected this view. The experiments herein summarized do not make possible an explanation of the phenomena, but they supply data which may suggest further lines of study leading to one.

For the study of conductivity, a clamp established compression between auricle and ventricle of an excised, quiescent, atropinized heart, or at the apex of a split ventricular preparation. Induction shocks were sent in by hand or by means of a special stimulator. Simultaneous electro- and mechanograms recorded conduction time. No simultaneous comparison of the excitability or contractility of the compressed muscle was possible. For these latter properties the changes in the ventricular excitability were followed while the contractions were recorded. The strength of the break induction shock was varied by changing the primary resistance, and the shock applied by way of non-polarizable electrodes.

Excitability, conductivity and contractility behave similarly in that: 1. Each displays a staircase. Conduction times become progressively shorter; the threshold progressively lower; the contraction progressively higher for the first several or more responses following a period of quiescence. 2. The increment of each is greatest for the first step of the staircase, and becomes progressively smaller thereafter. 3. Within limits, with increasing fatigue of the muscle, the optimal interval between stimuli appears to shorten, i.e., the crest of the supernormal curve seems to come earlier and, with reference to the resting level, to rise higher. 4. As a corollary of the last statement, increasing fatigue, within limits, favors the appearance of the staircase.

Two striking differences in behavior are to be noted, however: 1. Neither excitability nor contractility rises after prolonged rest periods. Conductivity shows the most marked depression after rest intervals of 15 to 30 seconds, with subsequent gradual improvement. 2. The staircase in excitability and contractility is always demonstrable under the conditions of our experiments; that of conductivity frequently fails to appear. We believe that the injury of the compressed muscle, and the probability that excitability is not the only factor influencing conduction through

[1] Adrian, E. D. Journ. Physiol., 1920, liv, 1.

physiologically non-homogeneous muscle, are responsible for these differences.

*The influence of certain vitamin deficiencies on gaseous exchange in chicks. I. Rachitis.* F. M. BALDWIN, V. E. NELSON and C. H. MCDONALD.

It appears from our study that the respiratory quotient (Haldane method) of the normally growing small chick between the weights of 75 grams and 500 grams is at a level somewhere between 0.94 and 1.00. This furnishes us with evidence that the chick in the normal economy of life processes, consistently utilizes carbohydrates while upon a diet adequate for protein, carbohydrates, fats, inorganic salts, and vitamins. Upon being deprived of an adequate supply of vitamin D, the chick is able for a while to continue the utilization of carbohydrates as is evidenced by the persistence of the respiratory quotient at the approximate level of unity. If the chick is kept for a considerable period of time upon a diet lacking in vitamin D, the characteristic symptoms of rachitis or so-called leg weakness will appear. Immediately prior to the appearance of such symptoms, however, a pronounced fall in the level of the respiratory quotient is noted. This fall is of a nature which would seem to indicate a rapid loss in ability to utilize carbohydrates as a chief source of fuel and heat and the substitution therefor of a mixture of carbohydrates and fats. Exhibition of the characteristic symptoms of leg weakness is attended by a respiratory quotient indicative of the utilization of fats alone although carbohydrates compose as high as 47 per cent of the diet upon which the chick is fed. The temperature of the chick falls slightly and remains low throughout manifestation of the symptoms of rachitis. There is but slight loss of appetite. Restoration of the antirachitic substance, either by the inclusion of cod liver oil in the diet or by irradiation, is attended by prompt relief of the characteristic symptoms of leg weakness. It is also attended by the rapid resumption of a respiratory quotient to the level approximating unity, hence indicative of ability to utilize carbohydrates for combustion. It is evident, therefore, that the presence of the antirachitic factor is requisite to normal metabolism in the chick and that it is definitely concerned with the ability of the chick to utilize carbohydrates.

*The $CO_2$ absorption curve of whole blood from dogs subjected to physical hyperthermia.* M. GARCIA BANUS and J. M. WILCOX.

Hyperthermia was produced in dogs under chloretone anesthesia by submerging all but the head of the animals in a bath at 41 to 42°C. The pharyngeal temperature of the animal was maintained at 40 to 41°C. for at least 6 hours. Blood samples were taken from the carotid artery before the onset of hyperthermia and at 2 to 3 and 6 to 6½ hours after. Clotting was prevented by use of heparin. In all cases, the animal survived for at least one hour after the last sample was taken. Controls were made on animals who received the same dose of anesthetic but were kept at 37°C.

In these controls, the $CO_2$ absorption curves determined from the blood at different intervals after the initiation of anesthesia were practically parallel to one another, although there was a shifting towards the acid side. A similar shift towards the acid side was seen in the $\frac{pH}{pCO_2}$ curves. These two facts seem to indicate that the anesthetic caused a reduction of the alkaline reserve.

In the blood of the animals that were made hyperthermic the following results were obtained: *a*, a shift in the $CO_2$ absorption curve towards the acid side, corresponding to that observed in the controls; *b*, a definite and very marked change in the shape of the curve so that in many cases it crossed the normal curve (i.e., that found in the blood drawn before hyperthermia). This change of slope occurred in some cases only after 6 hours of constant hyperthermia while in others it was observed as early as 2 to 3 hours; *c*, corresponding changes were observed in the $\frac{pH}{pCO_2}$ curves. These changes were accompanied, in all but one case, by a concentration of the blood as indicated by the total solid content. In all cases in which it was determined, there was observed a reduction in the hemoglobin content as determined by the $O_2$ capacity.

These results seem to indicate that in blood from animals subjected to at least 2 hours of hyperthermia, not exceeding 41°C. the following changes are observed in the whole blood when studied at a standard temperature (i.e., 25°C.): 1, a change in the apparent dissociation constant for $H_2CO_3$; 2, an increase in the buffering power of the blood for $CO_2$.

*The effect of local changes in temperature on the tensions of $O_2$ and $CO_2$ in the subcutaneous tissue.* H. C. Bazett and L. Sribyatta.

The observations of Goldschmidt and Light[1] on the effect of external temperature on the oxygen saturation of venous blood, as well as those of Stadie and Martin, and of Austin and his co-workers[2] on the effect of temperature changes on blood in vitro, have made it important to determine the effect of temperature changes in vivo on gas tensions. The development of a modified gas analyser for dealing with small volumes of gas has allowed the application of Campbell's[3] technic to this problem.

Nitrogen or alveolar air was injected subcutaneously in the forearm or hand in volumes of 7 to 50 cc. and analysed on removal one or two hours later in volumes of 0.3 to 0.5 cc. X-rays showed the depth of the gas to vary between 2 and 10 mm.; it was present in a thin irregular layer spread over a large area; the mean depth was about 5 mm. The arm was immersed in water at varying temperatures and the air removed within 5 to 10 minutes after withdrawal. Observations have been made of the actual temperatures existing at this depth under such conditions, by the use of McGlone loop thermocouples. Blood was taken in many experiments from a hand vein for comparison with the results of the gas analysis.

The $CO_2$ tension gives an approximately straight line when plotted with the temperature of the water as abscissa, and at ordinary skin temperatures, $CO_2$ was absorbed by the blood in the skin from arterial alveolar air. The $CO_2$ tension was 20 mm. below that of the alveolar air at a bath temperature of 10° and 5 mm. above it at 43°. This corresponds with a change in $CO_2$ tension of about 1.3 to 1.5 per 1°C. change in *tissue* temperature, and is of the same order as that calculated for the venous blood of these subjects.

The oxygen tension showed great changes; in subject B with a high

[1] Goldschmidt and Light. This Journal, 1925, lxxiii, 146.
[2] Austin and Cullen. Hydrogen ion concentration of the blood in health and disease. 1926.
[3] Campbell. Journ. Physiol., 1926, lxi, 248.

oxygen saturation of venous blood, the tissue tension varied between about 87 mm. in a bath at 42° to 24 mm. in one at 10°; in subject S, with a much lower oxygen saturation, it varied between 76 and 19 mm. Both subjects showed a particularly low level (S 14.5 mm.) at a bath temperature of 22°, when the venosity of the blood should be maximal according to Goldschmidt and Light. The temporary rise of oxygen tension at a still lower temperature must indicate that the high oxygen saturation of the blood depends on an incapacity of the tissues to utilise oxygen at such temperatures.

*Observations on volume-flow of blood in various parts of the body with varying alveolar gases and with the administration of sodium cyanide.* THEODORE BERNTHAL.

Using the continuous electrometric method (Gesell and Bronk) measurements were made of the volume-flow of blood in various regions of the body of the dog: 1. Femoral and brachial arteries both with the nerve supply to the region intact and with nerve supply destroyed. 2. Superior mesenteric artery with nerve supply intact and with nerve supply partially destroyed. 3. Thoracic aorta under conditions such that the total output of the heart was being measured.

The volume flow in these vessels was measured under the following conditions: 1. High alveolar $CO_2$ pressure. 2. Low and high alveolar $O_2$ pressure. 3. Intravenous injection of NaCN. In all cases measures were taken to eliminate effects of changes in blood pressure.

The results observed were as follows:

1. Brachial and femoral arteries. With the innervation intact the change in volume-flow in the arteries were the same as those reported for previous experiments. With innervation destroyed, there was absence, upon administration of low alveolar $O_2$, of the initial decrease and the post-administrational increase in flow (described in THIS JOURNAL, lxxxiii, no. 2). Upon administration of NaCN, likewise, there was an absence of the usually observed initial decrease in flow.

Upon administration of $CO_2$, there was no difference in the direction of the changes with nerve supply intact or destroyed. In either case a decrease in the volume-flow of blood was observed.

2. Superior mesenteric artery. There was an increase in the volume-flow of blood in this artery upon administration of high alveolar $CO_2$, low alveolar $O_2$ or intravenous NaCN. In a large per cent of the experiments—especially upon administration of NaCN—the increased flow was preceded by a small initial decrease in flow. The changes in volume-flow in this artery were influenced to a greater extent than in arteries of other regions of the body by changes in blood pressure. In general—visceral flow resembles carotid flow.

3. Total volume-flow. High alveolar $O_2$ produced no demonstrable effect upon the total volume flow of blood as contrasted with flow in individual arteries. High alveolar $CO_2$ and low alveolar $O_2$ both produced increases in the total volume-flow. The changes in total volume-flow, however, were much harder to elicit than changes in flow in isolated regions of the body.

Intravenous injection of NaCN produced an initial decrease followed by an increase in total volume-flow. The changes corresponded to those observed in all other regions of the body.

*Rhythmicity of response to galvanic and reflex stimulation of nerve.* GEO. H. BISHOP.

In view of the prevalent notion that nerve tends to respond only at the make and break of a galvanic current of moderate strength, it seems of interest to examine what is happening in the nerve during the interval. As the stimulus is increased until the nerve responds rhythmically, the increment of current necessary to cause the second response is large, while that necessary to cause a third response is smaller, which suggests a decrease of the current preceding the second process.

During galvanic current flow from a constant potential below threshold, the irritability as measured by the strength of superposed shock necessary to sum to threshold, increases for about one sigma, then decreases materially, then remains practically constant. During the same current, a shock of constant strength and of just threshold value when applied alone, has an increasing effect for about one sigma, then its effect decreases for a like time, after which the height of the action potential is almost constant. The changes are not due to cathodal depression nor to refractoriness.

These three effects may all be explained if we assume that the nerve envelopes (connective tissue, myelin, etc.) block the current due to their polarization, the initial response to a maximum current through the axons taking place before these envelopes become polarized. The current through the axons becomes constant only after equilibrium is established, after which the nerve tends to respond with a rhythm depending upon its refractory state.

It can be demonstrated in reflex frogs and turtles that single shocks to afferent nerves, or shocks repeated at one-fifth second intervals, just above threshold, elicit electrical responses in individual muscles which consist of trains of waves resembling those from a galvanic current which has become constant. If we can explain away the differences between the initial and subsequent effects of a galvanic stimulus, as a function of nerve envelopes, this removes one objection to the view that the central rhythm may also involve a repetitive response of a nerve ending to a persistent excitation. The efferent *nerve fiber* as such may be responding repetitively to a persisting effect, at the synapse, of the afferent impulse. The same consideration should apply to stimulation of a nerve by a persisting excitation in a sense organ, where the repetitive response might be a function of the stimulus intensity and of the refractory phase of the nerve ending.

*On direct current resistance levels in Valonia.* L. R. BLINKS.

Former experiments with the large multinucleate cells of *Valonia macrophysa* from Bermuda showed that the protoplasm was a very good insulator (at low potentials), i.e., few ions passed with a direct current. In cells of *V. ventricosa* freshly gathered at Tortugas the past summer, however, the resistance of the protoplasm was much lower, and there was a definite passage of ions at the same potentials as used before (15 to 25 millivolts potential drop along the cell). At slightly higher potentials the resistance increased, and rose sharply at above 50 millivolts to double or more the lower value. In some cases this amounted to nearly complete impermeability to ions, but usually fell below such a level. The effect was also reversible, low potentials again causing a greater flow of ions. Occasionally a cell would pass over from this variable resistance to a constant high level at all potentials, or be found at first in this condition, to become variable

during experimentation. The cause of this variability is uncertain, and is under further study.

At very high potentials, 200 millivolts or more, the resistance is in all cases lowered, breakdown and death resulting from long applications of high potentials. This is irreversible.

This divergence from Ohm's law suggests a polarization effect, but the study of opening and closing circuits, with an Einthoven galvanometer in the bridge to follow quick changes, indicates strong differences from ordinary polarization. A more likely cause seems to be the formation of a non-conducting structure under the influence of a polarization gradient. There may be a surface film formed when sufficient ions accumulate. Experiments with cells injured at one end showed the increase of resistance to occur only when the normal end was positive. Thus an excess of certain cations or deficiency of certain anions may cause the formation of a non-conducting surface.

The question remains whether the entire protoplasm varies, or whether the inner vacuolar surface remains impermeable to ions and the outer surface varies. Thus the thin film of protoplasm would offer a path for current at low potentials but become more or less completely cut out by the formation of a non-conducting outer surface at higher potentials. The numerical relations strongly suggest this effect. Several criteria can be applied to determine its possibility.

*The dual control of respiration.* EMIL BOGEN.

The acidity theory of regulation of pulmonary ventilation fails to explain variations in rate of respiratory movements. Acidity of the respiratory center, or increased carbon dioxide in the inspired air increases the depth of breathing without affecting the rhythm. Lack of oxygen is a specific accelerating factor affecting the rate of breathing. Both acidity and anoxemia indirectly affect each other, but their primary effects are independent. Other influences, both nervous and humoral, also affect the rate of breathing, both in health and disease, in man and animals, but the oxygen supply is of paramount importance.

*Studies on the surface area of the body in the developmental period of man.* E. BOYD, A. D. KLEIN, JR. and R. E. SCAMMON.

A series of determination has been made of the surface area of the body in the fetal period, infancy and childhood. Twelve determinations were made on fetuses ranging in age from the third lunar month to birth. Eleven were made on children. The twelve determinations for prenatal life were made directly from preserved specimens by coating the body with a flexible, non-shrinking mixture, which was removed in segments, traced on kodaloid, weighed and reduced to terms of area.

The eleven determinations for childhood were made from children ranging from three years and three months to four years and eleven months in age. Normal, living children were used in this study. A plaster cast was made of each child, and three determinations of the surface area of each cast were made by the kodaloid method. The growth in surface area of one child was followed for eight months by consecutive casts. The experimental errors involved in all the procedures were checked, experimentally and statistically, and were found to be extremely low.

The children were also weighed and measured for the various dimensions required in all combinations of the Du Bois linear formula for surface area. The areas as computed from these figures have been compared with the observed areas, as have also the computed values for surface area by the Meeh formula and its various modifications, and the formula of Drabkin and Cowgill.

A statistical study, based on 7000 seriatim determinations, from 35 normal children, has been made of the reliability of measurements of stem-length, sitting-height, body-length and standing-height, in connection with the estimation of surface area. The reliability of these measures falls in the following order: height, length, sitting-height and stem-length. The various types of sitting-height are about 50 per cent more variable than height, total or standing height, and stem-length is almost twice as variable as height, total or standing.

On the whole, the Du Bois length-weight formula, fits our observations more closely than any other, although some of the modifications of the Meeh formula approximate them.

Simple and semi-rational expressions have been developed for the relation between surface area and body length in early life. Birth seems to have little effect on the fundamental relationships of surface area.

*Glycolytic enzyme of the pancreatic juice: II. Experiments in sterile conditions in vitro.* EPHRAIM B. BOLDYREFF.

In previous publication was given sufficient evidence of: 1. Glycolytic action of pancreatic secretion in vivo—the decrease of the blood sugar due to secretion of pancreatic juice, and the increase of sugar content of the blood due to absence or deficiency of pancreatic secretion. 2. Glycolytic action of pancreatic secretion in vitro—splitting of the glucose by pancreatic juice.

Filtration of pancreatic juice through Berkefeld filter in my former experiments was responsible for the diminished glycolysis and low production of lactic acid. The technique was therefore modified; pancreatic juice was collected in sterile conditions and the experiments were also carried out in sterile conditions as was verified by bacteriological tests. The following glucose solutions of highest purity were used: 10 per cent, 1 per cent, 0.3 per cent and 0.2 per cent; 5 to 10 cc. of glucose solution and 0.5 to 1 cc. of pancreatic juice was used for each test. All specimens were incubated at 37.5°C.

The intensity of glycolysis was estimated by the amount of titratable acidity as well as by quantitative determination of glucose (Folin-Wu).

Sterile pancreatic juice possesses a strong glycolytic action. The amount of glucose split in the process was 40 per cent to 50 per cent. Thus, the existence of glycolytic enzyme in the pancreatic juice is confirmed by the experimental evidence.

*The pancreatic triad.* W. N. BOLDYREFF.

Our former experiments done on dogs have shown that a complete pancreatectomy and also severe loss of pancreatic juice through a fistula of the main pancreatic duct involve the following three pathologic conditions which we named "The Pancreatic Triad:" 1, a rise in the percentage of the blood sugar (i.e., decrease in the content of glycolytic ferment in the

blood); 2, a decrease of the blood coagulability—apparently due to decrease in the content of fibrin-ferment in the blood; and 3, appearance of leucocytosis. The duration of all these phenomena varies in different cases from several hours to several days or more.

Our new experiments on many dogs and observations on several men have confirmed former ones and have shown that this pathological triad may also be a consequence of other defects in the function of the pancreas. Thus after a partial pancreatectomy we observed a temporary appearance of all three members of the triad. Then we observed the triad as a consequence of the ligation of both pancreatic ducts on dogs; even a temporary closing of both pancreatic ducts with clamps may produce the same phenomena. Finally, we met with the mentioned triad in the cases of diabetes in dog and man.

We do not consider the name "triad" as a definitive term for the complexity of all observed by us pathological phenomena connected with the affection of the function of the pancreas. Their number can increase later. Thus we know that removal of pancreas or affection of its function involves usually a disorder of general metabolism and sometimes that of the fat-metabolism in particular.

*Pancreatic triad*

The figures obtained on dogs

| | NORMAL | DIABETES |
|---|---|---|
| 1. Blood sugar in per cent | 0.100 | 0.615 |
| 2. Leucocytes | 6.000 | 15.400 |
| 3. Blood coagulation time in seconds | 30 | 120 |

*Further observations on the diuretic substance of experimental diabetes insipidus.* HELEN BOURQUIN.

The following additional properties have been demonstrated in the diuretic substance present in the blood and in extracts of the mammillary bodies and adjacent tissue of the brains of dogs suffering from experimental diabetes insipidus. It is precipitated from solutions made weakly alkaline with barium hydroxide; is insoluble in ether, acetone, and ethyl alcohol; is completely destroyed by ashing; and is labile in solutions strongly alkaline even at room temperature, and in neutral extracts of the mammillary bodies allowed to stand at room temperature presumably due to enzyme action in the latter case.

The freshly secreted urine of dogs suffering from experimental diabetes insipidus, which has been evaporated to a small volume, treated with ten or more volumes of cold ethyl alcohol to remove the toxic substances, redissolved and filtered to remove the proteins, causes a prolonged diuresis accompanied by pronounced thirst when injected intravenously into normal dogs. Control injections of urine secreted over the same period of hours by a normal dog of the same weight, in which diuresis has been induced by giving water per orum, causes a comparatively slight and very transitory diuresis unaccompanied by thirst. This substance, which is present in the urine of diabetic but not of normal dogs, at least not in comparable amounts, is presumably identical with the diuretic substance present in extracts of the mammillary bodies and in the blood of these dogs for it possesses the same properties.

A diuretic substance also having these same properties has been demonstrated in extracts from the mammillary bodies and adjacent tissue taken from the brains of freshly slaughtered hogs, sheep, and cattle, but not in extracts of similar quantities of tissue selected at random from other portions of the same brains. The substance is present in comparatively small amount for the extract from five to six hundred hog brains produces about the same degree of diuresis as that which is caused by the extract from the mammillary bodies of the brains of three to five diabetic dogs.

*Physiological action of hydrogen sulfide on protoplasm of amoeba.* FLOYD J. BRINLEY.

In this study an attempt was made to determine whether the toxicity of $H_2S$ is due to its action on the cell membrane or on the internal protoplasm. Two species of amoebae were used—*Amoeba proteus* and a smaller species of the same group. The results obtained with the two species are essentially similar. The concentrations of $H_2S$ used, were saturated $\frac{3}{4}$, $\frac{1}{2}$ and $\frac{1}{4}$ saturated aqueous solutions. pH values of the solutions varied from 5.0 to 5.4. Some individuals were immersed in the solutions of $H_2S$ and others were injected with the same solutions by means of Chambers' micromanipulator.

When amoebae are immersed in aqueous solutions of $H_2S$, the viscosity of the protoplasm is increased, the animals swell slightly and assume a spherical form. The protoplasm collects into a semi-gelled mass, near one side of the membrane. A hyaline area occupies the remainder of the cell. Finally, the cell membrane ruptures in one or more places and the hyaline portion escapes into the surrounding solution. The hyaline fluid does not mix with the $H_2S$ solution but remains suspended as minute droplets.

Aqueous solutions of $H_2S$ were injected into amoebae in amounts equal to nearly one-half the volume of the cell. The sulfide quickly diffuses throughout the protoplasm and produces a temporary increase in viscosity. The organisms recover if the injection is not large enough to injure the cell membrane.

If amoebae are immersed in aqueous solutions of $H_2S$ and the membrane torn, a new membrane is formed over the injured surface.

The experiments seem to indicate that the toxicity of $H_2S$ for amoebae is largely due to its action on the surface membrane.

*Opposing action of $NaHCO_3$ and $NH_4Cl$ on the spontaneous activity of an esophageal preparation.* ERNEST H. BRUNQUIST.

Work of M. H. Jacobs, M. M. Brooks, Robert Chambers, H. W. Smith and others has afforded evidence that living cells are probably differentially permeable to a certain extent to the cation of $NH_4Cl$ and to the anion of $NaHCO_3$, and that these ions can produce intracellular hydrion changes which are more or less independent of the hydrion concentration of the environing medium.

A study was made of the effect of the two salts on the spontaneous activity (during two or three days) of mucosa-denuded preparations of frog esophagus. An apparent antagonism between the effects of the two salts was demonstrated. For example, a M/100 solution of $NH_4Cl$ in unbuffered Ringer's solution finally abolished the activity of the tissue. Ringer's solution with $NaHCO_3$ restored typical activity,—even though

it had been in abeyance for thirteen hours in one case. Such a procedure usually could be repeated at least twice with a given piece of tissue. Plain unbuffered Ringer's would not restore typical activity. The pH of the solutions used was kept within the range of 7.5 to 7.8 for the most part.

If the deductions from the work of the investigators mentioned above be accepted, the work herewith reported may be considered to afford provisional evidence of an intracellular antagonism between the cation of $NH_4Cl$ and the anion of $NaHCO_3$, unless the cell surface be shown to be the critical point in the activity.

*Does methylene blue itself penetrate living cells?* MATILDA MOLDENHAUER BROOKS.

Living plants of the marine alga *Valonia* were placed in buffered solutions of methylene blue at various pH values for several hours. The sap was then extracted and analyzed by means of the spectrophotometer. It was found that the dye present in the sap was methylene blue itself and not one of its lower homologs, as for example, trimethyl thionine. However, when the expressed sap containing the dye was allowed to stand several hours, the oxidation of methylene blue into trimethyl thionine was demonstrated spectrophotometrically.

*Bioelectric potentials in Nitella.* S. C. BROOKS and S. GELFAN.

By means of two microelectrodes stationed one just within the protoplasmic layer and one just outside the cell wall of the coenocytic cells of a species of *Nitella* it was possible to measure a bioelectric potential difference. This was found to increase as the size of the cells studied was increased from 0.5 to 3 mm.$^3$, the fluid surrounding the cells being tap water or 0.001 N NaCl. The corresponding potentials ranged from 10 to 40 mv. approximately, the electrode outside the cell being negative to that inside.

When cells were immersed in artificial *Nitella* sap, about 0.1 total normality, the potential was reduced to about 7 mv.; if this fluid was diluted before use higher potentials were observed regularly approaching with increasing dilution the potentials found for cells of equal size bathed by tap water. These results are qualitatively such as are to be expected if the potential is due to diffusion of electrolytes through a membrane, e.g., a protoplasmic surface, which offers on the average more resistance to ions of one sign than to those of opposite sign.

*Age factor in the production of bradycardia in experimental jaundice.* WILLIAM C. BUCHBINDER.

A marked slowing of the heart occurs in puppies 4 to 7 weeks old soon after they are made jaundiced by ligation and division of the common bile duct. The most acute slowing of the heart occurs during the first five days of obstruction when it may be slowed from an average of 200 to 150 beats per minute. It becomes progressively slower from this time to the 20th or 30th day when it usually reaches its lowermost level. After this there is an acceleration of the heart, the acuteness of which almost parallels the slowing seen in the initial stage of obstruction. A relative tachycardia usually persists from this time until death. The animal becomes dyspneic even in the absence of ascites, the heart tones become muffled and systolic murmurs are frequently heard. The electrocardiogram reveals an inver-

sion of the T-wave in the first and second, second and third, or in all leads, in addition to the bradycardia.

Bradycardia in the adult jaundiced dog was not encountered in a single instance. A slight degree of acceleration of the heart was the rule.

Incision into and release of bile from the gall bladder of the decerebrated frog produces almost invariably a sudden marked slowing of the heart with marked increased force of the beats. This may be prolonged over a period of 15 minutes or more before the normal rhythm is reëstablished. Simultaneous electrocardiographic and kymographic tracings of the suspended frog heart frequently reveal the almost immediate inversion of the T-wave of the electrocardiogram in addition to a marked slowing and arrythmia. Destruction of the medulla, decapitation, or atropinization prevents the reflex or abolishes it once after it is established. Marked distention of the gall bladder is noted in frogs in which the common bile ducts have been previously ligated and divided for several days. Heart block with sudden halving of the rhythm is frequently seen in these animals.

The slowing of the heart in jaundiced puppies is thought to be a specific reflex similar to that noted in the frog. It is believed that the mechanically distended intra- and extra-hepatic ducts serve as a focus for visceral sensory stimulation, and that the reflex is through the vagus. The absence of this reflex in adult animals may be explained on the basis of a less sensitive vegetative nervous system.

The tachycardia observed in the later stages of obstruction is thought to be the result of a poisoning of the myocardium.

*Diuretic-antidiuretic effect of the pressor principle of the posterior lobe of the pituitary gland.* E. P. Bugbee and A. E. Simond.

It has been demonstrated by workers in this laboratory that the posterior lobe of the pituitary gland contains two active principles, one which contracts uterine muscle and the other which raises blood pressure. We were interested in finding out if the diuretic-antidiuretic effect is associated with one of these or is due to still another active principle.

Experiments have been made on rabbits under urethane, amytal, chloralhydrate and chloretone anesthesia. It was found that the oxytocic principle has little effect upon the flow of urine, determined by counting the drops falling from a cannula in the bladder. The pressor principle causes first a decrease in urine flow, then a copious flow of short duration followed by diminished excretion. The relative intensity and duration of these three effects vary in different rabbits and depend upon the depth of anesthesia and upon the dosage of pressor principle.

Renal function tests on rabbits have been made in a manner similar to the commonly used clinical phenolsulphonephthalein test. It has been found that the oxytocic principle has little effect upon the excretion of dye whereas the pressor principle diminishes the rate of excretion of the dye.

*Study of a substance in desiccated tissues which increases sugar utilization more than insulin.* W. E. Burge, A. M. Estes and G. C. Wickwire.

The effect of a great number of substances on sugar utilization such as the optically active and optically inactive amino acids, aminoids, ovarian

and testicular substances, insulin, pituitrin, thyroxin, adrenalin, alcohol, inorganic salts, ether, chloroform, nitrous oxide and ethylene has been studied.

This investigation is concerned particularly with the effect of commercially prepared desiccated tissues such as aminoids, a commercially prepared meat digest with an amino nitrogen of 7.5 per cent, amino acids, desiccated testicular and ovarian substances. One hundred milligrams of each of these materials, as well as varying amounts of insulin, were added to 100 cc. of a 0.1 per cent dextrose solution in beakers. Two gold fish with a combined weight of five grams were introduced into each of the beakers and air was kept bubbling through the liquid to insure an adequate supply of oxygen. Sugar determinations were made immediately and after thirty hours. It was found that the aminoids produced a greater increase in sugar utilization than the amino acids taken singly or collectively. The desiccated ovarian and testicular substances greatly increased sugar utilization. Desiccated thyroid increased sugar metabolism while thyroxin did not. Insulin increases sugar utilization but to a less extent than the desiccated tissues. The view is advanced that none of these desiccated tissues per se increases sugar utilization but that they contain a substance which is responsible for the stimulating effect on sugar metabolism.

*Action of ultraviolet rays on limax flavus linn.* EMMETT B. CARMICHAEL.

The eggs from the nocturnal animal, *Limax flavus* Linn., which contained living embryos, were treated with the rays from a quartz mercury vapor lamp. They were not from a single animal but were collected from several places for each treatment. The collections of the eggs were made over a period of several weeks.

The eggs containing living embryos were divided into three classes:

1. Those having a large amount of pigment and thus almost ready to hatch.
2. Those with pigment in their eyes.
3. Those having no pigment but which were definitely developing.

Each of the above classes was treated and the time of treatment varied from 1 to 25 minutes. Treatments of 5 minutes or longer were quite deleterious to the embryos having a large amount of pigment, while those having no pigment could withstand the light for 5 minutes without any apparent gross effects.

Since the eggs were from more than one animal and since they were laid at different times, it seems that the results obtained are quite conclusive that eggs, from nocturnal animals, containing living embryos are not able to withstand long exposure to ultraviolet light.

*The energy changes of skeletal muscle accompanying contraction under high pressure.* MCKEEN CATTELL and D. J. EDWARDS.

We have previously described a method for the study of the effects of hydrostatic pressures, up to 80 atmospheres, on the rhythm and contractility of cardiac muscle. In the present experiments the same general technic for applying the pressure was employed, but the apparatus has been remodeled so that measurements may be made of the heat and the tension developed by skeletal muscle during activity. A pair of gastrocnemius

muscles of the frog are arranged in the pressure chamber in contact with a sensitive thermopile, and with their tendons attached to an optically recording torsion wire isometric lever. A uniform tension is maintained on the muscles by means of a special adjustment, and they are excited at stated intervals by single induction shocks applied to the sciatic nerves. The pressure chamber is filled completely, either with liquid petrolatum or Ringer's solution.

The application of a uniform pressure of sixty atmospheres upon a muscle results in an immediate increase in the tension it developes in response to a maximal stimulus. This augmented capacity for developing tension while under compression shows, in ten experiments, a range between 15.4 per cent and 63.4 per cent; with an average increase for the series of 33.3 per cent. When the pressure upon the muscle is restored to the original atmospheric level, the capacity for developing tension similarly returns to its pre-pressure value. In a given preparation intermediate grades of pressure acting upon the muscle give proportional increases in the amount of tension it developes when stimulated.

Simultaneous measurements of the initial heat production in muscles subjected to pressure exhibit a close agreement with the tension changes. The myothermic measurements for ten experiments, under a condition of 60 atmopsheres compression, show an average increase of 31.7 per cent over the pre-pressure series. By rapidly applying pressure to a muscle and releasing it in a similar manner, we have shown that the augmenting effect that compression exerts upon heat and tension is immediate and fairly stable.

*Non-protein nitrogen during muscular exercise.* WILLIAM H. CHAMBERS and HERMAN YANNET.

In previously reported experiments it was found that moderate exercise on a treadmill produced in dogs an increase in nitrogen excretion during the first week of fasting. This extra urinary nitrogen was spared by feeding carbohydrate or exhausted by prolonged fasting and therefore may not be a direct product of muscular contraction.

For this reason the first step in the blood studies on this problem has been to compare the non-protein nitrogen of the blood from resting and working leg muscles. In dogs under amytal anesthesia one hind leg was exercised by applying an induced tetanizing current twice per second. Twelve cubic centimeter blood samples were drawn simultaneously from the exposed femoral veins of the resting and contracting legs and immediately afterwards from the femoral artery, at three different times during the exercise. Besides the N. P. N. of the whole blood the specific gravity was also determined by the falling drop method. The total nitrogen excreted in the urine was measured for the preliminary, work, and post work periods.

According to controls on blood and urea solutions the method is adequate to show differences in N. P. N. equivalent to 0.3 or 0.4 mgm. per 100 cc. of blood. The biological variation of the individual blood samples is within the limits of the method in some experiments, in others somewhat greater.

The following table illustrates the results obtained:

| | TOTAL NITROGEN IN URINE | | | N.P.N. IN WHOLE BLOOD | | |
|---|---|---|---|---|---|---|
| | Pre-work | Work | Increase | Arterial | Venous—resting leg | Venous—exercising leg |
| | *mgm. per hour* | *mgm. per hour* | *mgm. per hour* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* |
| Dog 211 | 159 | 205 | 46 | 32.3 | 32.5 | 32.3 |
| Dog 96 | 78 | 109 | 31 | 22.6 | 22.3 | 22.6 |
| Dog 96 | 91 | 120 | 29 | 24.0 | 24.1 | 24.3 |

The N. P. N. figure is in each case an average of the 3 blood determinations during an exercise experiment. The difference between the venous blood from the resting and exercising legs varies between +0.3 and −0.2 mgm. Approximately the same difference is found between venous and arterial blood. The specific gravity data show no significant change in blood concentration.

*A non-specific pressor substance.* J. B. COLLIP.

Extracts have been prepared from a number of different tissues which have the property of producing a profound rise in the blood pressure when administered by intravenous injection to dogs or cats under amytal anesthesia. The pressor effect is not due to epinephrin or pituitrin. Extracts having this peculiar pressor effect have been prepared from the following materials: skeletal muscle, liver, kidney, spleen, ovary, testis, stomach, thymus, intestine, whole fetus, and several commercial preparations of both pepsin and pancreatin.

Potent pressor extracts may be prepared from any of the above mentioned tissues in a variety of ways. A satisfactory method is as follows. The tissue is thoroughly minced and extracted with water either neutral or acidulated at boiling temperature for five minutes. This simple extract is filtered and the filtrate is concentrated in vacuo until it is of a syrupy consistency. Absolute acetone is then added and the mixture shaken violently. On standing an acetone layer and a saline water layer separate. The acetone is removed and the saline layer is again extracted in a similar manner. This extraction process may be repeated three or four times. The acetone extracts are combined, filtered and concentrated in vacuo almost to dryness. The residue is again extracted with absolute acetone as in the first instance. The whole process is repeated as often as necessary to ensure removal of practically all inorganics and the major part of depressor substances.

The pressor principle is believed to be the same in all extracts made in the above method from the tissues mentioned. It is very difficult to secure complete removal of depressor substances without considerable loss of the pressor principle. The pressor principle is soluble in absolute alcohol, and in mixtures of acetone, ether and water in various degrees. Thus a solution of the principle plus adherent substances in acetone, ether and a trace of water will on the addition of water separate into two layers, the water layer containing both pressor and depressor substances whereas the ether-acetone layer is practically free of depressor substances.

The pressor principle is soluble also in mixtures of absolute alcohol and petroleum ether and may be fractionally precipitated therefrom by the addition of petroleum ether.

The potency of active extracts may be entirely lost under certain conditions which are as yet not fully established. Acetic anhydride removes the pressor effect of extracts. Prolonged exposure in the air at high temperatures as on a water bath may be attended with loss of the pressor effect. Dilute solutions however appear to be uninjured by either acid or alkali in moderate concentration at boiling temperatures.

The active principle may be removed to a large degree by adsorption on norit at a pH of about 8. It may be released again by treating the norit with boiling water made acid to congo.

Intravenous injection of these extracts is followed after a latent period of a few seconds by a rather sudden rise in blood pressure. There is a concomitant increase in lung ventilation manifested as a rule both in increased rate and depth of breathing. Return of blood pressure to normal is an orderly process, the time involved being dependent upon the dosage administered and the peak point of blood pressure attained as indicated in the records.

The pharmacology of the active principle is a matter of considerable interest. This has yet to be worked out in detail. In preliminary experiments with isolated heart,intestine and uterus, small amounts of the active extracts are practically without effect, while large doses are depressant.

The administration of cocaine by the intravenous route causes a decrease in the pressor effect of a definite dose, if not a complete abolition of the same. The pressor response returns rather soon and in the course of an hour may be back almost to the original level.

Atropine is without effect upon the pressor response of a unit dose.

Gynergen on the contrary produces marked enhancement of the pressor response of a unit dose. A moderate dose of nicotine may produce slight but definite enhancement in the pressor effect.

Experiments to determine the degree of central action of the principle have yet to be made. If a relatively large dose is administered there are as a rule very definite phases of vagus inhibition manifest in the blood pressure tracing. Intravenous injection of these pressor extracts is well tolerated by normal rabbits. There is little or no effect upon sugar concentration in such experiments.

It has been observed that a pressor extract obtained from the prostate glands of oxen is not antagonized by cocaine as are extracts obtained from the sources mentioned above. This is being investigated further. It would appear however that the prostate gland yields a pressor substance which has a somewhat different pharmacology from the non-specific pressor substance described herein.

*Some factors influencing the normal isometric contraction period of the human heart.* O. M. COPE.

Résumé of previous work done in attempting to determine the exact length of the isometric period.

Method: Synchronous optical records of carotid tracings and heart sounds, as suggested by Wiggers.

Factors modifying the length of the period: position; sex; blood pressure; age; heart rate; pathological circulatory conditions.

Conclusion: Approximate normal length of the period; normal modifying factors; pathological modifying factors; possible applications of these data in diagnosis and prognosis of cardiac and other circulatory abnormalities.

*The excretion of dyes by the pancreas.* LATHAN A. CRANDALL.

This work is a continuation of that of Oldberg and Ivy,[1] who studied 32 dyes. Only two dyes of their series, namely, methylene blue and methylene violet, were excreted by the pancreas.

The method used is similar to that of Oldberg and Ivy. The main pancreatic duct is cannulated and a balloon on the anterior abdominal wall is connected to the cannula by means of a Rehfuss tube. Pancreatic secretion was stimulated by food or water, and 40 to 150 mgm. of dye in distilled water or in physiological salt solution were injected intravenously. The juice was collected at hourly intervals for 2 hours and then at irregular intervals for 24 hours.

The present series comprises 62 dyes. Only those which have been excreted by the pancreas will be mentioned. They are acid fuchsin, fast yellow, new fuchsin, methyl orange, methylene violet (previously studied by Oldberg and Ivy), para-fuchsin, rhodamine B, safranin O, and tropaeolin O. Three of these belong to the azo group, two are safranins, three are rosanilins, and one is a rhodamine. Representative dyes from all other groups with the exception of one have been studied, but none were excreted. Four of the excreted dyes (acid fuchsin, fast yellow, methyl orange, and tropaeolin O), can be recognized only when the pancreatic juice is acidified. Safranin O and methylene violet are mildly toxic, para-fuchsin is slightly toxic, and the others are non-toxic in the doses used.

No property common to the dyes which the pancreas will excrete and not present in other dyes has been discovered. They have been considered on the basis of chemical structure, acid or basic properties, lipoid solubility, and molecular size.

Since no dye has been found to be excreted in concentration sufficient to serve as a function test of the pancreas, this series will be extended in the hope of finding such a dye.

*Changes in the electric potential of tissues in living animals under normal and pathological conditions.* GEORGE W. CRILE, AMY F. ROWLAND and MARIA TELKES.

Measurements were made on living animals, to show potential differences between the subcutaneous fascia and other injured and uninjured tissues including the cerebrum, liver and muscle. It was found that a certain potential difference exists and that this depends on the general condition of the animal and on the anesthetic used.

When the animal was subjected to different harmful influences such as irritation of the nerves, trauma, insomnia, asphyxia, hemorrhage, etc., potential-difference measurements showed certain characteristic variations.

When certain physiological agents were injected intravenously, such as adrenalin, strychnin, NaCl, KCl, $MgSO_4$, $CaCl_2$, NaCN, morphin (subcutaneously), etc., potential-difference measurements again showed characteristic and reproducible variations.

[1] Oldberg, E. and A. C. Ivy. Soc. for Exper. Biol. and Med. (Proc.), 1927, xxiv, 838.

It was found that while the animal was dying, the potential difference dropped to a minimum value but increased again a few minutes after death and again dropped to a final zero value in a few hours.

The conclusion is drawn that this experimental method is a valuable means for the detection of physiological changes in the living organism.

*Uniformity of narcosis in peripheral nerve.* H. DAVIS and L. H. RICE.

Most of the evidence supporting the theory of "conduction with a decrement" in narcotized nerve has been satisfactorily explained without the hypothesis of decrement. But the increase in the least interval for muscular summation with increasing length of narcotized nerve, as described by Lucas, was denied by Kato,[1] but later confirmed by Cooper.[2] We have sought an explanation for this contradiction in terms of unequal narcosis of the nerve.

The time required for narcosis of a 12 millimeter length of various parts of the sciatic nerve of the bull-frog is not clearly related to the diameter of the nerve.

We find no evidence for more rapid narcosis of the upper thigh as compared with the lower thigh region of the sciatic nerve in small frogs. For narcosis 8 mm. lengths without branches were selected.

Careful and complete removal of all adherent fascia usually results in an earlier narcosis of the nerve so cleaned. This is probably due to the slight mechanical trauma involved.

Cooper's and Kato's experiments on the least interval for muscular summation were repeated. Kato's results were confirmed in six experiments on bull-frogs in which branching regions of the nerve were avoided. The least interval increased equally at all points. If branching regions of the nerve were used, results more or less like Cooper's were obtained, but in only three of seven experiments was correspondence perfect. The others were intermediate, part of the nerve following one type, part the other. All of our results can readily be explained on the hypothesis that the narcotic acts more rapidly at the point of junction of a branch with the main trunk than on an unbranched region. This is in agreement with Kato's[3] recent experiments on this point, and further confirms the theory of "decrementless conduction."

*The comparative sensitiveness to epinephrin of blood pressure and intestinal motility in man.* C. A. DRAGSTEDT and J. W. HUFFMAN.

We have previously reported that in the unanesthetized dog pressor effects may be obtained on sustained injection of epinephrin in the absence of intestinal inhibition.[4] Dianelopolu, Simici and Dimitriu[5] reported that in man 1 cc. of 1 to 300,000 adrenalin exaggerated the peristaltic contractions of the small intestine but that 1 cc. of 1 to 100,000 produced an inhibition. They did not report simultaneous observations on blood

[1] Kato, G. 1924. The theory of decrementless conduction in narcotized region of nerve. Tokyo, Japan.

[2] Cooper, S. 1926. Journal of Physiology, lxi, 305.

[3] Kato, G., K. Nakajima, D. Teruuchi and K. Kanai. 1927. Keio Medical Science, vii.

[4] Dragstedt, C. A. and J. W. Huffman. Proc. Soc. Exper. Biol. and Med., 1928, xxv, 241.

[5] Dianelopolu, Simici and Dimitriu. Compt. Rend. Soc. Biol., 1925, xcii, 1143.

pressure. Csepai[1] reported that as a result of a large experience with intravenous injections in man the average patient gave a rise of 20 to 30 cm. of water with 1 cc. of 1 to 100,000 epinephrin. No simultaneous observations of intestinal motility were reported.

We have made simultaneous observations of blood pressure and intestinal motility in man after injection of epinephrin and have seen blood pressure rises of 15 mm. in the absence of any effect on intestinal motility.

*Relation of parathyroidectomy to voluntary activity in the white rat.* E. P. DURRANT.

Observations by means of revolving activity cages on both unilaterally and bilaterally parathyroidectomized rats ninety to one hundred days old show that though tetany is equally pronounced after both operations the activity is merely temporarily depressed; but the recovery is much more rapid following the unilateral ablation. In rats two hundred to two hundred and ten days old the results are the same qualitatively but are more variable in amount.

*Effect of desiccated thyroid feeding on growth of the guinea pig.* E. P. DURRANT.

Thyroid feeding experiments were made on seven litters of guinea pigs extending from 12 days to 3 months from birth. The dosage was $\frac{1}{4}$ grain desiccated thyroid (Armour's) on alternate days with some and the same amount once a week with others. The heavier dosage was followed by the death of the animals in from 12 to 20 days but all the thyroid-fed animals grew less rapidly than their litter controls; the former showing an increase of weight of 12 per cent to 480 per cent, the latter, an increase of from 75 per cent to 675 per cent.

*Vasomotor responses to perfused adrenalin in the unanesthetized dog.* R. R. DURANT and J. H. MCNINCH.

The spleens of two young female dogs were externalized by the method described by Barcroft and Stephens. After a short recovery period during which the incisions had healed completely, the volume changes of the spleens were recorded by means of an oncometer strapped to the abdominal wall. Blood pressure was recorded separately and also simultaneously with the spleen volume, by the direct method. The cannula was inserted in the femoral artery under local novocain anesthesia. Intravenous injections of minimal doses of adrenalin (Parke, Davis & Co.) were found to produce only rises in blood pressure and constriction of the spleen. These observations confirm those of Dragstedt and lend support to the "emergency" theory of suprarenal function.

*The blood pressure of adrenalectomized rats.* R. R. DURANT.

The adrenals were removed from 20 female rats, approximately two years old and varying in weight between 157 grams and 400 grams. The blood pressures of 11 of these animals determined by the direct method within the first 5 hours following the operation varied considerably with extremes of 85 mm. and 145 mm., and an average of 112 mm. Hg. The

[1] Csepai, K. Adrenalinempfindlichkeit Innere Sekretion und Vegatatives Nervensystem, 1924.

last figure has been found previously to be the average pressure for normal rats of the same age. The blood pressures of the remaining 9 animals, determined 6 hours to 22 days after the extirpation were low with a minimum of 52 mm., a maximum of 95 mm. and an average of 77 mm. Hg.

Ten animals of the same age and of both sexes were operated as controls. Two had one adrenal removed, another had one whole capsule and all but a small piece of cortex of the other, removed. A sham operation simulating the adrenalectomy was performed upon the remaining seven. The pressures of this group were found to vary between 65 mm. and 135 mm. with an average of 104 mm. Hg. Eliminating the data obtained upon 2 animals which at autopsy proved to be in very poor condition the average pressure for the control group is 111 mm. Hg. The three animals that had undergone partial adrenalectomy possessed the normal pressure.

These observations indicate that in rats adrenalectomy has no immediate effect upon blood pressure, but that after 6 hours a marked fall in pressure occurs and that the adrenals through either their cortical or medullary components are concerned in the maintenance of normal pressure.

*The influence of thyroidectomy on tissue respiration.* J. A. DYE and R. A. WAGGENER.

Plummer,[1] Kendall[2] and others have suggested that the action of the thyroid hormone on the tissues of the body, with accompanying changes in metabolism, is probably catalytic in nature. The very marked delay in the action of thyroxin when injected intravenously into normal or myxedematous patients is hardly in accord with what we know regarding catalytic action. The same objection may be more strongly applied to an assumed drug-like action of thyroxin on the tissues. It occurred to us that this might not be a direct action of the hormone, but an indirect one in which the intracellular oxidizing systems are the immediate structures involved.

To test this assumption experimentally, we have examined certain tissues (heart muscle, kidney cortex, liver and skeletal muscle) from three cretin lambs (twelve weeks after thyroidectomy) and three cretin pups (four and one-half months after thyroidectomy—method of Dot), together with similar tissues from normal animals of the same age and sex (twins and litter mates), for their power to catalyze the oxidation of a-naphthol and dimethyl-paraphenylene-diamine-hydrochloride into indophenol blue. Quantitative determinations were made by an improved colorimetric method. All tests were carried out at room temperature which fluctuated between 22 and 25°C. These readings were later corrected for 23°C. which is the optimum for the indophenol oxidase. An appreciable amount of activity is still evident at 0°C. while the enzyme is completely inactivated at 55°C. in thirty minutes.

A marked diminution in the power of these tissues from thyroidectomized animals to catalyze the oxidation of the substrate was found in each case with the exception of liver. Higher values were obtained for this tissue in two of the three lambs while lower oxidation rates were found in all the tissues examined from the pups without exception. This diminution in amount, activity, potency or other property of the tissue oxidase of cretin

[1] Plummer, H. S. 1924. Journ. Amer. Med. Assoc., lxxxiii, 1333.
[2] Kendall, E. C. 1919. Endocrinol., iii, 156.

lambs, as compared with that of the control animal taken as unity, averaged 15 per cent for heart, 45 per cent for kidney, 20 per cent for liver and 50 per cent for skeletal muscle in 20 minute readings. Lower values were obtained for the pups, in which the tissues were affected more equally, viz., heart 16 per cent, kidney 13 per cent, liver 21 per cent and skeletal muscle 9 per cent. These figures compare favorably with the well known fact that basal metabolism may be reduced 30 to 40 per cent below normal in cases of hypothyroidism.

*Changes in the tonicity of smooth muscle produced by toxins of Ascaris lumbricoides.* F. E. EMERY and C. A. HERRICK.

In the isolated contracting smooth muscle, marked changes in tonicity were produced by toxins of ascarids obtained from the pig. Segments of the intestine of a rat, placed in Locke's solution in a constant temperature bath, showed an immediate rise in tonus when small amounts of the aqueous ascaris extracts were added. This rise in tonus was accompanied by an increase in the rate of rhythmical contraction of the segment. The amplitude of the contraction does not seem to be correlated with the amount of the extract added. Similar results were obtained with the isolated intestines of the cat, rabbit and guinea pig.

The above reactions may occur in the intestine of the living animal. This was shown by certain experiments with dogs, rats and guinea pigs to which the toxins were administered through a stomach tube. In these cases some colicky symptoms were observed and feces were passed at frequent intervals thus indicating an increase in peristalsis.

*The effect of the inorganic salts on sugar utilization.* A. M. ESTES, E. WILLIAMS and W. E. BURGE.

It is known that the organic food materials, proteins, fats, and sugars stimulate metabolism. The object of this investigation was to determine the effect of the inorganic salts on sugar metabolism specifically. The salts used were the chlorides, sulphates, carbonates, and phosphates of calcium, potassium, sodium and magnesium. Two hundred milligrams of the following were introduced into beakers containing 100 cc. of 0.1 per cent dextrose, di-sodium phosphate, calcium di-phosphate, sodium bi-carbonate, calcium carbonate, calcium chloride, sodium chloride, magnesium chloride, sodium sulphate, and magnesium sulphate. One hundred and fifty milligrams of di-potassium phosphate, potassium bi-carbonate, potassium chloride, and potassium sulphate were dissolved in other portions of 100 cc. of 0.1 per cent dextrose. Two gold fish with a combined weight of approximately 5 grams were introduced into each beaker, and air was kept bubbling through the solutions to insure an adequate supply of oxygen. The reason for using smaller amounts of the potassium salts was their greater toxicity. Two hundred milligrams of the potassium salts killed some of the fish in the experiments. Sugar determinations were run immediately and at the end of thirty hours of the solutions in the beakers using Benedict's method. In four series of experiments the controls used on an average of 33 per cent of the sugar. Only three of the salts affected sugar utilization. Magnesium sulphate produced a decrease of 12 per cent which is attributed to the anesthetic effect of this salt. Di-potassium phosphate produced a very great increase, 68 per cent against 33 per cent

for the control. Di-sodium phosphate produced 46 per cent increase against a control of 33 per cent. It is known that the sodium salts in the form of chlorides occur principally in the liquids of the body while the potassium salts in the form of sulphates are found principally in the solids, muscle cells, for example, where sugar utilization is greatest. It is assumed that the function of the potassium phosphate in the cells is to increase sugar utilization there.

*Estimation of heart weight and volume in the dog.* J. A. E. EYSTER.

The constitution of an approximate normal for heart weight and volume in the dog is of importance because of the frequent employment of this animal in studies in experimental cardiac hypertrophy and because heart weight estimation by comparison with simple body measurements (heart weight body weight and heart weight vertebral length ratios) shows such wide divergence. The present study is an effort to make this prediction with greater accuracy, which has been successful only to a limited degree at present. Various body measurements and a frontal plane teleroentgenogram were obtained from 185 dogs during life and the heart removed and weighed immediately after the animals were killed. Of the various simple ratios, including only one measurement compared with heart weight. the only one found to offer a distinct improvement over the heart weight, body weight ratio was a direct estimation of the heart weight from the area of the frontal plane teleroentgenogram. If one plots the individual cases on distribution curves, showing the extent of departure from the predicted expressed in terms of the predicted, it is found that, using the mean heart weight body weight ratio (0.007478) as a standard, 47 per cent of all cases depart more than $\pm$ 10 per cent and 28 per cent by more than $\pm$ 15 per cent from the predicted. In the case of other ratios, and this is true of the comparison between heart weight and x-ray area, the ratio varies with the magnitude of the variable, and it is necessary to plot one function against the other and to obtain a value for the criterion for each magnitude of the variable from the regression line drawn through the plotted cases. Using this method for prediction of heart weight from frontal plane x-ray area, the departure from the predicted is slightly but definitely reduced. Cases falling beyond a $\pm$ 10 per cent range now comprise 43.5 per cent and those beyond a $\pm$ 15 per cent range 22 per cent. By statistical treatment of the data and predicting from a regression equation employing the variables of higher relative weight, these figures have been still further reduced to 36 per cent and 17 per cent respectively. The variables employed in this case were six, body weight, girth and transverse diameter of the thorax, length of sternum and plane area and greatest transverse diameter of the teleroentgenogram. An attempt is being made to still further improve the accuracy of prediction by taking account of certain curvilinear functions in the mathematical treatment.

Heart volume can be more accurately predicted than heart weight at present, by employing the formula proposed by Skavlem, in which heart volume equals $0.44\ A^{3/2}$ when A is the area of the frontal plane teleroentgenogram. This method has been tested in 23 cases in the present series. The actual volume in most instances approximates closely the predicted and all fall within a $\pm$ 10 per cent range. Heart weight predicted in these animals on the basis of body weight and by the regression equation

employing six variables showed 56 per cent and 31 per cent respectively beyond the ± 10 per cent range and 26 per cent and 17 per cent respectively beyond the ± 15 per cent range.

*Simultaneous and continuous determinations of oxygen and carbon dioxide in nerves and muscles during activity.* W. O. FENN and D. S. MARTIN.

Oxygen is determined as usual in a respirometer by volume. Carbon dioxide is absorbed in barium hydrate and determined by conductivity. A frog sartorius muscle is suspended in such an apparatus in contact with a thermopile so that heat production can also be followed. When the muscle is stimulated the initial heat is measured and during recovery the increased gas exchange is found sufficient to account for the recovery heat but insufficient to burn more than a fraction of the lactic acid equivalent of the initial heat. When the muscle goes into rigor both the resting heat production and the gas exchange increase together.

Nerves can be stimulated in a similar apparatus in which arrangements are also made for recording the negative variation. Previous results can be confirmed by this method. When nitrogen is administered the negative variation ceases when the anaerobic carbon dioxide formation falls below about 0.05 cu. mm. per gram per minute. The nerve may be left in nitrogen for 8 hours and still recover almost completely when oxygen is admitted. During this recovery both the oxygen consumption and the carbon dioxide output pass through a maximum indicating both an oxygen debt of 10 volumes per cent and an oxidative removal of accumulated products. The oxygen debt may be due in part also to an oxidation reserve. These experiments therefore confirm and extend the observations of Gerard and Meyerhof who observed a much smaller oxygen debt over shorter periods of anaerobiosis but found no evidence of oxidative removal.

*Concerning the fate of calcium after its injection into normal animals.* A. FINE and N. B. TAYLOR.

With the view of throwing some light upon the hypercalcemia of parathormone administration as well as upon the lowering of the calcium in tetany, an effort was made to discover the fate of calcium after its injection into normal animals.

It was found that when an amount of calcium was injected sufficient to raise the blood calcium from the normal of about 10 mgm. to over 25 mgm. per 100 cc., from 70 to 80 per cent of the injected calcium disappeared within 3 hours and could not be accounted for. Attempts were made to trace this loss through the small and large intestines and the kidneys.

The experiments were performed in the following way. The normal excretion of calcium through the intestine of cats (large and small bowels separately) was first ascertained. The large bowel was ligated at its junction with the rectum and just above the ileo-colic sphincter. It was first thoroughly washed out until the final washing was calcium-free. The intestine was replaced within the abdomen and the animal allowed to recover from the anesthetic. Three hours later the segment of bowel was again washed out until the washings were free of calcium. The total washings were saved and the amount of calcium therein estimated. The total normal excretion into the large bowel was found to be from 0.15 to

0.33 mgm. In the case of the small bowel the same procedure was followed but in most cases only a segment of the bowel—about a quarter of its length—was isolated. The total excretion varied from 0.16 to 0.38 mgm. over a 3-hour period.

In another series of animals 60 mgm. of Ca per kilo, in the form of $CaCl_2$, were injected into the femoral vein after the large bowel or a segment of the small was washed out so as to remove thoroughly all calcium from the lumen. The Ca of the blood rose from approximately 10 mgm. to between 25 and 30 mgm. in 100 cc. In 3 hours this had dropped again to a little above the normal—13 to 14 mgm. At the end of this period the total amount of calcium in the washings was from 0.14 to 0.25.; that is, practically the same as when no calcium was injected. In one case the excretion through the entire length of the small bowel was estimated after calcium injection. It was found to be closely comparable in amount to that excreted through the small segment.

The removal of the kidneys or the liver did not alter the rate at which the injected calcium disappeared from the blood. Practically none of the calcium which disappeared can be accounted for by excretion through the intestines or kidneys.

*Some physiological effects of copper.* FREDERICK B. FLINN.

In studying the physiological effects of copper under closely controlled conditions, we have found that copper is not retained in the body except in small amounts. The elimination takes place in the gastro-intestinal tract. Experiments with the gall bladder show that some of the metal is absorbed into the body but is filtered out by the liver and excreted in the bile. Copper is distributed in small amounts all over the body, the amount depending on the vascular supply, but is found chiefly in the liver. We have examined the livers of numerous animals and have found copper in each case. An examination of 33 human livers from persons having no copper exposure showed copper contents of from 5 to 15 mgm.

Although copper forms the carbonate and phosphate salt at the same hydrogen ion concentration as lead, it is not found in the bones, except in traces.

Copper is found in normal blood as well as in the blood of animals that have been exposed to copper other than that contained in their natural food. An examination of the blood of exposed animals shows that the copper is divided between the plasma and corpuscles, approximately in the ratio of two to one. My experiments with blood *in vitro* indicate that one cannot draw correct conclusions as to what one may expect when salts are ingested in a normal manner, in experiments where metal is injected intravenously or subcutaneously. During the investigation with blood *in vitro* I found that some methemoglobin was being formed. This does not take place when animals are fed copper. A spectrophotometric test does not indicate the presence of methemoglobin. The blood of our copper-fed dogs was further examined by the Van Slyke-Neill method, in which sodium hydrosulphite is used to reduce methemoglobin to hemoglobin in one sample of blood called *A*. A second sample called *B* was run without the addition of hydrosulphite. Both samples were saturated with illuminating gas and the difference in the results between *A* and *B* should have given the amount of methemoglobin present in the original blood. *A* in this case

should be higher than *B*. A summary of part of the work is indicated in the following table:

| | *Sample A* vol. per cent | *Sample B* vol. per cent |
|---|---|---|
| Normal before feeding | 26.95 | 27.16 |
| 2 weeks' copper feeding | 27.12 | 31.02 |
| 2 months' copper feeding | 31.00 | 31.74 |
| 3 months' copper feeding | 32.09 | 34.35 |
| Animals fed lead | 23.88 | 23.90 |
| Animals fed aluminum | 25.74 | 25.67 |
| Lobster's blood | | |
| No. 1 | 2.49 | 2.59 |
| No. 2 | 3.05 | 3.04 |

An examination of the blood of guinea pigs which were fed copper for 18 months showed a continued increase in the hemoglobin content of the blood over a control group. The findings on the pigs and dogs show that the blood of copper-fed animals has an increased oxygen-carrying capacity over animals that are not fed copper.

It has been shown that no other metal than copper forms two oxides and two chlorides in which the combination is of so loose a character. The combination of CO and CUCL is a loose reversible union. Apparently part of the copper in the blood is acted upon by the hydrosulphite and is prevented from acting or uniting with the CO. The evidence of our work so far indicates that the copper is present in the body partly as a catalytic agent, but that its reaction is readily interfered with by impurities.

*"Fatigue" phenomena in the peroneal nerve and the flexion reflex in the cat.* A. FORBES and R. W. GERARD.

The frog's sciatic is known to exhibit a form of fatigue ("equilibration") when tetanized continuously for some minutes. This appears to depend largely on a prolonged refractory period. In the present work the cat's peroneal has been stimulated with several hundred shocks a second for two to six minutes and the action currents recorded with the string galvanometer. A falling off of response, often to a regular submultiple of the stimulating frequency, is noted. Rise of threshold is an important factor in producing this effect.

Even with very slow tetanization, the individual action currents show marked changes as a result of activity.

The flexion reflex of the decerebrate cat, in response to single shocks, shows a decline when repeated at sufficiently brief intervals (measured by the electric response in the peroneal nerve). This had been explained in terms of persistent activity of some of the motoneurones after the first reflex discharge, rendering them refractory to the afferent impulses of the next reflex—the "line-busy" effect. Some doubts have arisen regarding this interpretation, and the duration of "line-busy" effect was directly determined by the following method. A maximal direct shock is applied along the course of the intact peroneal nerve (the lower end of the nerve is cut and arranged on leads to the galvanometer) at varying intervals after a similar shock has been applied to the central end of the ipsilateral popliteal, thus producing a reflex response along the peroneal. As long

as any fibres in the peroneal continue to carry impulses following the reflex stimulation the direct stimulus will not affect them, and the response to the direct stimulus will be correspondingly reduced. This "line-busy" effect could not be demonstrated when the interval between stimuli was more than 50 sigmata. The decline of successive reflex responses will still occur when they are elicited at intervals of 0.8 second or longer. Some factor besides "line-busy" effect, therefore, must play a rôle in the reflex paths, and this may be similar to the "fatigue" in the axons.

*The energy metabolism of cattle in relation to the plane of nutrition.* E. B. FORBES, WINFRED W. BRAMAN and MAX KRISS, with the collaboration of C. D. JEFFRIES, R. W. SWIFT, ROWLAND B. FRENCH, R. C. MILLER and C. V. SMYTHE.

A series of experiments was conducted, with steers as subjects, both by the methods of direct and indirect respiration calorimetry, for the study of the energy metabolism in relation to the plane of nutrition, and related problems.

Five planes of nutrition were studied with each of two steers. As indicated by the quantity of feed given, these planes were 1, fast; 2, half of the maintenance requirement; 3, maintenance (energy equilibrium); 4, half more than maintenance; and 5, twice the maintenance requirement.

The subjects were two Aberdeen-Angus steers, between 25 and 33 months of age; and throughout the study the results with the two steers agreed phenomenally well.

The heat production increased but little from fast to the plane of half of the maintenance requirement, and increased much more rapidly from the latter plane of nutrition to the maintenance level; but the entire rise in metabolism from the fasting to the maintenance level was at a distinctly lower rate than was the fairly uniform increase from the level of maintenance to that of twice the maintenance requirement.

The relation between the heat production and the food consumption, above maintenance, therefore, is expressed by a nearly straight line, and, below maintenance, by a pronounced curve.

The several factors which determined the curve of heat production in relation to the plane of nutrition seem to be 1, the amount of the observed heat production of fast; 2, the energy cost of utilization of food; 3, the difference in the energy cost of utilization of food for body increase as compared with the utilization of the same for the prevention of body loss; and 4, the influence of the incompleteness of digestibility and metabolizability of the food on the proportions of the same which actually contribute to the heat production.

The curves of distribution of feed energy per kilogram of dry matter between feces, urine, methane, and heat increment, and digestibile, metabolizable, and net energy, as affected by the plane of nutrition, were plotted.

From the plane of half of the maintenance, to that of twice the maintenance requirement, these curves were characterized as follows:

Digestible energy first rose slightly, on account of increased digestion of crude fiber, and decreased, at an increasing rate, as a result of lowered digestion of carbohydrate and protein.

The curve of energy of the feces, naturally, was the reverse of that of digestible energy.

The curves of methane and urine energy were nearly straight lines, falling slightly with rise in plane of nutrition.

The curve of total heat increment (computed with reference to the fasting ketabolism) rose at each period of observation, but each time at a decreased rate of rise.

At the maintenance level of nutrition, 17.5 per cent of the heat increment was found, by the use of Krogh's factor, to consist of heat of fermentation of carbohydrates.

The curve of total net energy (for maintenance and body increase together) was a practically straight line, falling rapidly with rise in plane of nutrition.

The curve of total heat increment was separated into components for maintenance and for body increase, that for body increase (computed with reference to the heat production at maintenance) being much higher than that for maintenance (computed with reference to the fasting katabolism).

The results of this study signify that there must be separate net-energy values of feeds for maintenance and for body increase; and that to such extent as the earlier net-energy values of this institute were derived from submaintenance feeding periods, they are mixed values.

The net-energy values of the ration, with the two steers, were, at maintenance, 2218 and 2241 Calories; at half more than maintenance, 1550 and 1557 Calories; and at twice the maintenance requirement, 1531 and 1557 Calories.

From this study it appears that comparable determinations of the specific dynamic effects of feeds, foods, or nutrients, can be determined only at the same plane of nutrition.

*Some clinical observations upon the physiology of the hypothalamus.* J. F. FULTON and PERCIVAL BAILEY.

Careful clinical study of circumscribed hypothalamic lesions strongly supports the belief that important "vegetative" centers exist in this region. In one patient a small tumor confined chiefly to the tuber cinereum *without involving the hypophysis* was associated with marked diabetes insipidus, amenorrhea, adiposity, and recurrent attacks of drowsiness—all in the absence of symptoms of increased intracranial pressure. From an analysis of a group of such cases it is evident that disturbances confined to the hypothalamic centers may in fact cause genital dystrophy with adiposity, diabetes insipidus, recurrent attacks of drowsiness similar to normal physiological sleep, and curious mental states which for want of better term we have designated emotional negativism. Whether any or all of these symptoms are due to the interruption of nerve impulses normally passing from the hypothalamic nuclei down the infundibulum to the pituitary is left open, but, save possibly for the diabetes insipidus, this explanation is looked upon as improbable. We concur in the belief that an area of grey matter exists in the posterior part of the hypothalamus, disturbance of which leads to hypersomnia, but we think it improper to refer to this, as some have done, as the "sleep center."

*The recording of single nerve action potentials with the cathode ray oscillograph and the analysis of a volley from the central nervous system into a phrenic nerve.* HERBERT S. GASSER.

Heretofore it has been possible to see the tracing of a single wave of nerve activity on the screen of a Johnson cathode ray oscillograph, but not to record it. It has now been found that by two very simple changes in technique the necessary conditions for recording can be obtained. The first change was made at the suggestion of Dr. William Dock and consisted in the use of Eastman duplitized x-ray film. The second was in an increase of the anode-cathode voltage above the ordinary working range.

As previously, the time axis was controlled by a condenser discharge; but to avoid fogging of the more sensitive film the line was made of sufficient length to leave the screen of the tube on both sides. However, even when the resting beam did not strike the screen, with the greater voltage the fluorescence from the scattered electrons was sufficient to darken the film, if it were left on the screen any length of time. Accordingly, the anode-cathode circuit was closed immediately before the spot was released and broken immediately afterward. Two break keys controlled the release of the spot and the position of the stimulating shock.

Single waves from green frog nerve of the dimensions commonly observed with the standing wave technique could be readily recorded when a driving voltage of 450 was used.

The fastest potential change to be recorded in nerve physiology is the axon action potential in mammalian nerve at body temperature, which lasts about $0.6\sigma$. This was recorded from the desheathed phrenic nerve of the dog at 38°, leading from the stimulating cathode. The fastest record which was still legible was obtained with 465 volts. In the time line $1\sigma$ was spread over 6.4 cm. The figure was over 3 cm. high and had a spread at the base of 3 cm., dimensions more than sufficient to meet any experimental need, and necessitating an equivalent linear spot velocity of 10 cm./$\sigma$.

The new technique allows the employment of the Braun tube in the study of nerves activated from the central nervous system. Leads were made from the phrenic nerve using three or four panel amplification, and the individual volleys were analysed. While formerly the only attribute of the discharge of the nervous system which could be analysed was the frequency, in the Braun tube records the height and area of the waves also have significance and afford additional information. The details of these experiments will be published in the near future.

*Further observations on respiratory control.* ROBERT GESELL.

Part I. Peripheral and central chemical control of ventilation. Experiments were performed on the dog bearing on the relative importance of peripheral and central stimulation of respiratory movements by intravascular administration of sodium cyanide.

Injection of cyanide sufficient to excite respiratory movements when administered intravenously may be ineffective when injected directly into the carotid artery. If the opposite carotid is clamped the cyanide may now be effective. Clamping of one or both vertebral arteries increases the effectiveness still more. These results seem explained by the shunting of the cyanide blood to the respiratory centers. Clamping of the internal carotid prevents the stimulating action of cyanide. Injection into the vertebral arteries is the most effective. Since no stimulation is obtained when the internal carotid is clamped it is assumed that peripheral chemical stimulation in the head region is of little significance in respiratory control.

Part II. Lymph acidity and lymph flow. Administration of carbon dioxide increased the acidity of the thoracic duct lymph. Sodium carbonate and bicarbonate produced the opposite effects. The administration of sodium bicarbonate was sometimes accompanied by an initial acid change.

The administration of sodium cyanide and of low oxygen invariably increased alkalinity of the blood and lymph. This was frequently followed by an increased acidity above the normal value. The initial increased alkalinity was smaller or missing if constant artificial ventilation was maintained.

The lymph was found to be about 0.1 pH more acid than the blood. The administration of $CO_2$, low $O_2$ and cyanide increased the flow of lymph which was followed by subsequent retardation during recovery. The increased turbidity of the lymph indicated increased permeability of the vascular membranes involved.

The results correspond in degree with those obtained under similar circumstances in the cerebrospinal fluid and support the views on the significance of the acidity of the interior of the respiratory neurones on respiratory control.

*Simultaneous observations on expired oxygen and carbon dioxide, blood acidity, blood flow, blood pressure, carbon dioxide capacity and lactic acid content of the blood with relation to pulmonary ventilation.* ROBERT GESELL, THEODORE BERNTHAL, GRACE GORHAM and HUGO KRUEGER.

Preliminary experiments showing effects of hemorrhage and intravenous injection of sodium cyanide, and sodium bicarbonate on expired oxygen. expired carbon dioxide, blood acidity, volume flow of blood, carbon dioxide capacity and lactic acid content of the blood were performed. The various changes in expired gas and blood analyzed with respect to disturbances in oxidations and acid base equilibrium in the tissues show apparent agreement.

In some few determinations blood from the right auricle contained more lactic acid than corresponding arterial blood. Such results indicate the removal of lactic acid from the blood by the lungs.

*The acidity of the cerebro-spinal fluid under a variety of conditions as related to respiratory control.* ROBERT GESELL and ALRICK HERTZMAN.

The administration of a gas low in oxygen by constant artificial ventilation was accompanied by no effect on acidity or by a slight increase in alkalinity. This was followed shortly during the anoxemia or on readministration of room air by a definite increase in acidity above the normal value. It the same gas was administered by normal ventilation the final acid effect was preceded by a definite alkaline change.

Administration of sodium cyanide during constant ventilation elicited an acid effect which was most pronounced on the return of normal or hyperoxidation. This acid effect was preceded by an alkaline effect when cyanide was administered during normal ventilation.

Hemorrhage during constant ventilation slowly increased the acidity of the cerebro-spinal fluid which was temporarily augmented by reinjection. The effects of hemorrhage and reinjection during normal ventilation were more uncertain.

Since an ultimate increased acidity of the cerebro-spinal fluid from low alveolar oxygen, cyanide and hemorrhage were associated with an increased ventilation it is assumed that the increased ventilation may be primarily related to increased intra-cellular acidity. Since an immediate effect of these administrations on the acidity of the cerebro-spinal fluid may be missing, slightly acid or definitely alkaline it is indicated that the acidity of the immediate environment is not the determinant factor controlling the volume of ventilation. But since these instances of unchanged or increased alkalinity of the cerebro-spinal fluid may be associated with an increased rate of respirations greater than occurs with the administration of carbon dioxide when the cerebro-spinal fluid turns distinctly acid the question of the possibility of the differential regulation of rate and volume of respiration should not go unconsidered. The possibility of a lowered state of oxidation of the neurone membrane as another rate augmenting factor should be analyzed.

*Latent tolerance in diabetes mellitus.* R. B. GIBSON.

Diabetics desugarized on maintenance diets with a F. A.: G of 1.5 and the required insulin have shown remarkable improvement following high sugar ingestion with increased insulin. Two to three days of high sugar ingestion with intervals of routine management in series increases the tolerance until dietary management without insulin may suffice to control the diabetes; the most severe cases may require a greatly diminished insulin dosage. The procedure is especially effective in younger patients.

*The effects of cold and of veratrin upon the electrical responses of ventricular strip preparations.* A. S. GILSON, JR.

The electrogram from a strip preparation of turtle ventricular tissue with leads placed near the two ends shows an irregular or completely doubled-peaked R-wave. The break is due to the interruption of fiber paths near the apex. Using three lead-off electrodes, one near either end and one at the mid-point in the six possible permutations of two at a time, with stimulating electrodes placed near each of the two end leads, the conditions of the strip at the regions near the three leads may be studied.

The break in the R-wave as it occurs in our records offers a check on local conditions in a specific sort of preparation. The interpretation of the interruption or change in direction of fiber paths is one which has general application in the study of electrocardiograms.

Veratrin (0.1 per cent in Ringer's solution) at first decreases the period of mechanical shortening, the times for conduction through the strip, the rise of the R-wave, and the duration of the total electrical response. With severe veratrin poisoning, all of the above times are very greatly increased. Between the two extremes is a condition in which the period of mechanical shortening, and the time of rise of the R-wave are normal or less than normal, but the duration of the total electrical response may be longer than normal. This is shown to be due to a delay in conduction which occurs at particular localities, especially at the apex. The degree of blocking at such a point may increase with the severity of the poisoning to an extent causing a delay of a second or more at the apical region before the establishment of complete block. Qualitative experiments have shown that calcium chloride may have a similar effect. With decreased

temperature the times between all points along the electrogram usually become increased in direct proportion.

It appears, therefore, that there are junction-like connections between different fiber bundles of ventricular tissue which normally present little if any delay to the passage of an impulse. Under the effect of certain poisons such as veratrin or calcium chloride these junctional points are especially affected, causing the delay or complete blocking of a transmitted impulse.

*The electro-uterogram as an index of the nature of the automatic contractions of the uterus.* CHARLES W. GREENE.

Electro-uterograms have been taken of the isolated automatically contracting uterus of the rat. These records show a positive deflection of the galvanometer coincident in time with the total automatic contraction, as simultaneously recorded in the myogram. The duration of this disturbance of electrical potential lasts from 30 to 40 seconds or longer.

Coincident with the contraction phase of the myogram there are rapidly recurrent and rhythmic electric deflections, the rate of these deflections is from 117 to 120 or more per minute, far more rapid than any demonstrated simple muscle contraction of smooth muscle tissue. The deflections themselves are slightly diphasic. They begin with a low potential difference, augment slightly through the first 3 or 4 deflections, remain relatively constant for 20 or 30 deflections, then become slower in periodicity and slighter in amplitude to the end of the series at the crest of the contraction phase. In a typical simple contraction there are from 25 to 36 electric pulses in this series.

The limitation of the electric impulses to the contraction phase argues in favor of the view that these are expressions of a stimulating mechanism. The further fact that the deflections begin before the mechanical contraction can be interpreted as supporting the same hypothesis. It is suggested that the electric deflections are too rapid to have their origin in smooth muscle tissue, and by exclusion we are at present supporting the hypothesis that the deflections are neural in origin.

*Does the feeding of cod-liver oil prevent tetany in thyroparathyroidectomized dogs?* ISIDOR GREENWALD.

The feeding of cod-liver oil for two or three weeks before thyroparathyroidectomy, and thereafter, did not protect dogs against tetany. This is contrary to the results reported by Jones.[1]

*The relationship between fibrin content and sedimentation index in tuberculous individuals.* ESTHER GREISHEIMER and OLGA H. JOHNSON.

This series of investigations was carried out on tuberculous patients between twenty and thirty years of age. It includes 51 women and 50 men. The sedimentation index was determined by the Cutler method and the fibrin by the Wu colorimetric method, with slight modifications. The fibrin determinations were done in duplicate.

The average sedimentation index for women was 17.000 mm. $\pm$0.769; the average index for men was 11.980 mm. $\pm$ 0.820. The difference

[1] Jones, J. H., Journ. Biol. Chem., 1926, lxx, 647.

between the two sexes is 5.020 ± 1.124. This difference is 4.4 times as large as its probable error, and is probably significant. The average fibrin content for women was 0.414 per cent ± 0.009. For men it was 0.435 ± 0.014. The difference between the two sexes is 0.021 ± 0.017. Since this difference is of the order of magnitude of its probable error, it cannot be considered significant.

Treatment of the sedimentation indices and the fibrin contents by the statistical method gave a correlation coefficient of 0.736 ± 0.0415 for the women. This is 17.7 times as large as its probable error, which shows unmistakable correlation between the sedimentation index and the fibrin content. When the empirical mean fibrin values as ordinates are plotted against the sedimentation indices, considering the relatively small number of cases, the agreement of these means with the theoretical means given by the regression line calculated from the correlation coefficient is satisfactory.

The correlation coefficient for men was 0.689 ± 0.0501, which is 13.7 times as large as its probable error.

The difference between the correlation coefficients for the two sexes is only 0.047 ± 0.0655. Since the difference is less than its probable error, it cannot be considered significant.

*Simultaneous determination of the greater and lesser circulation times, of the mean velocity of blood flow through the heart and lungs, of the cardiac output and an approximation of the amount of blood actively circulating in the heart and lungs.* W. F. HAMILTON, JOHN WALKER MOORE, J. M. KINSMAN and R. G. SPURLING.

By injecting an easily detectable substance[1] into the venous side of the vascular bed and analyzing successive samples taken every second or so from the arterial side, it is possible to plot a concentration curve of definite mathematical form whose descending slope fits the exponential equation $y = ae^{-bx}$ This curve shows prima facie landmarks which determine the greater and lesser circulation times.

Evidence from circulation models indicates that the data presented in the concentration curve can be used to determine the rate of fluid flow. Further evidence indicates that calculations of the cardiac output by the direct Fick method are not significantly different from the results of the injection method.

The pulmonary circulation time has been used by others to calculate the amount of blood in the lungs when the cardiac output is known. This calculation is based upon the assumption that the fastest particle of blood passes through the lungs at very nearly the average speed. That this assumption is unwarranted is seen from an analysis of the concentration curves, based upon careful determinations of the injected substance in successive samples of arterial blood taken every second or so. The time, measuring the average velocity of flow of the injected particles, can be shown to coincide with the time intersection of an ordinate dividing the area of the curve into two equal parts. This is obviously quite different from the time intersection of the beginning of the curve.

Experiments upon models indicate that calculations of the volume of

[1] Phenol-tetraiodo-phthalein sodium, Mallinckrodt.

the model thus based upon the average velocity, check the actual volume much more closely than they do when performed in accordance with the published formulae. This is true, whether the flow be through chambers, tubes, or chambers filled with beads to simulate flow through a capillary bed.

The detailed correspondence between the in vivo and in vitro curves justifies our using the same procedure to compute the amount of blood actively circulating in the heart and lungs. This quantity is closely related to the factors which govern the filling of the left ventricle.

*Physiology and pharmacology of the crop (esophageal) muscles.* P. J. HANZLIK and E. M. BUTT.

The anatomical arrangement and innervation of the esophageal muscle are described. Reciprocal responses of the circular and longitudinal muscles occur under different conditions, and appear to be invoked through the myenteric reflex. The sympathetic and parasympathetic innervations are both augmentatory; consistent with this are the actions of typical drugs. The pharmacology of several important groups of drugs is described; also reversals and antagonisms of contracted crop muscle by drugs which contract the same muscle, thus indicating the importance of the functional state in pharmacological actions and amplifying the experimental basis of certain therapeutic responses of smooth muscles. (Series of papers appearing in THIS JOURNAL and Journ. Pharm. Exper. Therap.)

*The respiration of luminous bacteria.* E. NEWTON HARVEY.

If an emulsion of luminous bacteria is allowed to stand undisturbed, the luminescence remains constant and then dims suddenly, due to the utilization of the oxygen dissolved in the sea water. Since the consumption of oxygen is independent of the oxygen concentration until dimming begins, we can calculate the oxygen consumption from the solubility of oxygen in sea water, by simply observing the time for dimming. This method and the Thunberg microrespirometer method were used with the same emulsion of luminous bacteria and found to agree within 1 per cent. The oxygen consumption at 21°C. is 0.29 cc. per gram bacteria (of $1.1 \times 2.2\ \mu$ dimensions) per minute. Using the formula, $C_o = \frac{Ar^2}{5D}$, which approximately applies to a short cylinder, one can calculate the oxygen pressure, $C_o$, at the surface of a bacterium, which will allow all parts of a bacterium of short radius, $r$, to receive enough oxygen, to respire at the rate observed, $A$. If the diffusion coefficient of oxygen, $D$, found by Krogh for connective tissue, is used, the oxygen pressure for adequate respiration comes out about one-quarter the observed pressure at the surface of a bacterium when its luminescence begins to dim. The latter value, approximately 0.26 per cent oxygen, was determined, in experiments with C. S. Shoup, by means of flow meters used to mix hydrogen and oxygen in various proportions. If the luminescence intensity is an index of respiratory rate, our results suggest that oxygen diffuses into a bacterium more slowly than into gelatin, muscle, or connective tissue. Comparison of respiratory rates of different tissues, per gram of tissue, are only of value if it is shown that the oxygen supply is adequate for complete penetration. Otherwise it is better to compare respiratory rates per surface area of tissue involved.

*Excitation at the anode in nerve by short induction shocks.* PETER HEINBECKER.

In the literature frequent references are made to repetitive action potential waves resultant from single induction shocks. With the cathode ray oscillograph it is possible to show that such action potential waves may result from stimulation at the anode by short induction shocks. Such findings have been obtained in freshly excised sciatic nerves of the bullfrog and green frog; also in nerve roots of the bullfrog.

The action potential wave arising from the anode is similar to that arising at the cathode. Its appearance is delayed by approximately the duration of the stimulus applied. Its origin at threshold is in different fibers from the excitation wave developed at the cathode. This is proven by the fact that on increasing the strength of shock with the cathode proximal to the galvanometer leads the anodal wave is blocked by the cathodal wave; on reversing the current the cathodal wave is blocked by the anodal wave. In irritable nerves it is possible to demonstrate repetitive anodal waves, one wave of the series following in the relative refractive period of the preceding one.

Using a constant current 0.1 sigma in duration and the voltage less than 2 volts, anodal stimulation at the break of the current is demonstrable. By separating the stimulating electrodes the anodal and cathodal action potential waves can be shown as separate and distinct processes.

These findings indicate the similarity of the induction shock to a constant current of short duration.

*Experiments on the peristaltic mechanism.* V. E. HENDERSON.

A series of experiments has been carried out during the past two or three years in an attempt to secure experimental information as to the mechanism of peristalsis. The first of these experiments served to show that the peristaltic mechanism is put into activity by increasing the internal pressure thus stretching the muscular coats. The mechanism is a local one. Any nerve connections involved must be short. Secondly, that the mechanism cannot be entirely myogenic. Thirdly that for the isolated gut the type picture of a peristaltic wave must be that of advancing waves of tonus on which the rhythmic movements are superimposed which is not preceded by an area of inhibition of movements, and probably not of tonus. The experiments lead to the belief that the peristaltic mechanism depends upon the intactness of short reflex arcs within the gut itself.

*Changes in lactic acid and glucose in the blood on its passage through organs.* H. E. HIMWICH, Y. D. KOSKOFF and L. H. NAHUM.

Changes in the lactic acid and glucose content of the blood on its passage through various organs have been studied in 40 dogs. The main source of lactic acid is muscle while the organ chiefly concerned with its removal is the liver. The heart may participate in the removal of lactic acid from the blood. Since the chief site for the production of the glucose of the blood is the liver there appears to be a carbohydrate cycle between muscle and liver, muscles sending lactic acid by means of the blood to the liver and the liver returning glucose via the blood to the muscles.

*Cyclic variations in the basal metabolic rate of women.* F. A. HITCHCOCK and FRANCES WARDWELL.

For the past year we have been investigating the normal variations occurring in the basal metabolic rate of women. Significant data are now available on some twenty different subjects. The basal metabolic rate of each subject has been determined two or three times a week. A Roth-Benedict apparatus was used and the technic that described and recommended by Roth. One subject has been under almost continuous observations for a year. Another has been similarly observed for nine months, while the data on the remaining eighteen cover periods ranging from two to six months. Fifteen of the subjects showed a lowering of the B.M.R. during the menstrual period, while in the other five there was either a slight rise or no change at menstruation. Usually a rise in the rate occurred during the week preceding the beginning of menstruation. In a number of cases a marked depression was observed about the fourteenth day after the beginning of menstruation. In some cases this depression was as great or greater than that which occurred during menstruation itself.

*Resuscitation from ventricular fibrillation.* D. R. HOOKER.

The ventricles of the dog's heart when caused to fibrillate by electrical stimulation do not recover spontaneously. A method has been sought which will induce recovery and which may be of practical usefulness in electrical accidents to men. Direct ventricular medication is without avail but treatment via the coronaries has a large element of promise. The procedure of choice at the present time is to cannulate the carotid and to inject, under pressure, 0.5 per cent KCl until the heart comes to rest (less than 50 cc. being required for a 4 kgm. animal) and to follow with a Ringer's solution, without KCl and with epinephrin added. The solutions are saturated with oxygen and warmed to body temperature. A number of hearts have been repeatedly recovered by this technique. A blood pressure tracing was shown from one animal which was allowed to recover and was found to be in good condition the next day.

*Studies on the glandula uropygialis of birds.* H. C. HOU.

The bilobular preen gland of fowls, pigeons, ducks and geese has been excised and the subsequent behavior of the animals compared with that of normal controls. Unless carefully separated the operated birds tend to steal oil from the glands of their normal relatives. Contrary to the opinion of previous workers, the operation frequently affects the well-being of the birds. It also has a marked effect upon the ease with which the feathers become wetted.

*The inorganic phosphorus of mammalian blood and muscle.* LAURENCE IRVING and G. M. BASTEDO.

The ordinary acid-soluble inorganic phosphorus of dog and cat muscle may be slightly increased after prolonged contraction, but the true inorganic phosphorus from decomposition of Fiske and Subbarrow's phosphocreatine may be increased, several times. In spite of this great increase in muscle inorganic phosphorus, that of venous blood from the muscle shows no certain change compared with arterial blood during the same

period. Increases in water and lactic acid of the contracting muscles indicate the magnitude of other metabolic changes, so that the blood and muscle are evidently not in equilibrium as regards inorganic phosphorus, which passes through an intramuscular cycle.

*Identification by spectrophotometer of the presence of trimethyl thionin in the vacuole of cells placed in methylene blue.* MARIAN IRWIN.

When uninjured cells of the marine plant *Valonia macrophysa* were placed in methylene blue dissolved in sea water, the blue dye was found to penetrate the vacuole and collect in the sap (more rapidly the higher the external pH value). When the blue dye penetrating into the sap from methylene blue solution at pH 9.5 was extracted from the vacuole and immediately analyzed by means of the spectrophotometer, it gave the absorption curve of a dye consisting chiefly of trimethyl thionin, with a primary absorption maximum at 650 m$\mu$, while the external methylene blue solution gave an absorption curve characteristic of a dye containing chiefly methylene blue with a primary absorption maximum at 665 m$\mu$. Methylene blue is found in the sap only when the cells are injured or only when contamination of the sap from the stained cell wall (which absorbs methylene blue) at the time of extraction of the sap from the vacuole takes place. The less trimethyl thionin there was in a sample of methylene blue solution the slower was the rate of penetration of blue dye into the vacuole.

When cells were placed in pure trimethyl thionin solution, the dye penetrated more rapidly the higher the external pH value. The dye thus collected in the sap gave an absorption curve characteristic of trimethyl thionin with a primary absorption maximum at 650 m$\mu$.

When the experiments were repeated with the fresh water plant *Nitella flexilis* the same results were obtained except in one respect. The dye collected in the sap of the vacuole of cells placed in the methylene blue solution at pH 9.5 gave an absorption curve characteristic of a dye consisting chiefly of trimethyl thionin with a primary absorption maximum at about 655 m$\mu$ which showed the presence of a greater amount of methylene blue than in the case of cells of *Valonia*. This difference may be due either to the greater penetration of methylene blue with *Nitella* as a result of slight injury or to the difference in the selective permeability of these cells to substances. It cannot be due to greater contamination from the cell wall during the extraction of the sap because about one-fourth of each cell of *Nitella* was left outside of the solution, covered by damp absorbent cotton. The sap was extracted from the end of the cell which had not been immersed in the dye solution.

Since every sample of methylene blue has a trace of trimethyl thionin, it would be unsafe to draw any theoretical conclusion on the permeability of cells or oxidation reduction potentials of cells based on the penetration of blue dye from methylene blue unless the nature of the dye inside and outside the cell were determined very carefully.

*A hormone mechanism for gall bladder contraction and evacuation: Physiological studies.* A. C. IVY and ERIC OLDBERG. *Chemical studies.* A. C. IVY, GRANT KLOSTER and H. C. LUETH.

We have found that an extract of the duodenal and jejunal mucosa can be made which, on intravenous injection, causes the gall bladder to contract

and evacuate. In a dog under light barbital or ether anesthesia with the cystic duct clamped and the fundus of the gall bladder cannulated, a single injection causes a rise in intra-gall bladder pressure of from 2 to 5 cm. of bile pressure. Tonus-rhythmic contractions may result amounting to as much as 2 cm. of bile pressure. Injections at five- or ten-minute intervals in a dog with the cystic duct cannulated, previously cannulated aseptically the day before, has caused a 6 cm. rise in pressure. The rise in pressure from one injection persists from 15 to 60 minutes, usually about 20 minutes. If the gall bladder is filled with lipiodol and then 18 to 24 hours later the animal is injected intravenously every ten minutes, for one hour, the lipiodol is evacuated from the gall bladder. In some cases 90, in other 60, and in others only 40 per cent of the lipiodol is evacuated. Only one failure has been observed and egg-yolk and cream were ineffectual in this animal.

N/10 HCl, 1 per cent butyric acid, digested egg-yolk and cream (pancreatin), when introduced into the duodenum with the cystic duct clamped and the fundus of the gall bladder cannulated, caused a rise in pressure and an increase in the tonus rhythm.

Four cross-circulation experiments have been performed in compatible animals with the cystic duct clamped, the gall bladder cannulated, and the pancreatic duct cannulated. An injection of N/10 HCl into the duodenum of one animal caused, after a latent period of from 8 to 12 minutes, the gall bladder of the other animal to contract. Three of the four experiments were positive. In only one experiment was there an increase in pancreatic flow.

We tentatively suggest the term "cholecystokinin" to designate the principle in intestinal extract which causes the gall bladder to evacuate.

The active principle occurs in the "new secretin" of Weaver, Luckhardt and Koch[1] and most of it remains in the non-absolute-alcohol soluble fraction. We have prepared solutions of "secretin" of which from 1 to 10 mgm. of solid material have caused changes in the gall bladder in 10 to 20 kilo dogs. Our process of purification has not been uniformly successful. If one extracts the intestine with 0.5 per cent $H_2SO_4$, by placing 200 to 300 cc. into the lumen of the freshly excised upper intestine after washing it with cold water and then adding 20 per cent trichloracetic acid to make a 5 per cent solution, the precipitate contains no dilatin, but about 90 per cent of the "secretin" and 50 or 60 per cent of the "cholecystokinin." The precipitate is then dissolved in hot water (90°C.), filtered clear when cold, and then evaporated to dryness. The residue is extracted with ether, absolute alcohol and 80 per cent alcohol. Usually all of the "secretin" goes into the latter fraction, which in some cases, has proven to be active in amounts as small as 0.3 mgm., of solid material when injected into 10 kilo dogs. The "cholecystokinin" for the most part remains in the 80 per cent alcohol insoluble fraction. Sometimes 50 per cent of it will go into the 80 per cent alcohol soluble fraction. We have made preparations by this method, as well as by other methods, which have caused gall bladder action, but no pancreatic action.

Although we believe, at present, that the gall bladder excitant is different from the pancreas excitant, our evidence is still meager.

If a thin-walled glass cannula about $1\frac{1}{2}$ inches long and $\frac{3}{8}$ of an inch in

[1] Weaver, Luckhardt and Koch. Journ. Amer. Med. Assoc., 1926, lxxxvii, 640.

external diameter, is inserted into the duodenal portion of the common bile duct through an incision in the latter so that ¼ of an inch projects into the lumen of the duodenum, and if lipiodol is placed in the gall bladder, a low tension clamp being on the common bile duct during this procedure and removed just before closing the abdomen, we have found that the lipiodal begins leaving the gall bladder sometimes before the animal is out from under the anesthesia and in the course of from 6 to 24 hours much of the lipiodol has left the gall bladder even though the animal has not received water or food. We believe that this shows that the duodenum determines whether or not the contraction of the gall bladder will result in evacuation.

*Differential relaxation during reading, writing and other activities as tested by the knee-jerk.* EDMUND JACOBSON.

The influence of relaxation on the knee-jerk was tested on 14 trained subjects during reading, writing and copying numbers. During relaxation the jerk was diminished in all instances as compared with the previous control period. In the period after relaxation as compared with during relaxation there was a significant increase of jerk in about one-half the cases.

No marked diminution of the jerk occurred when the subjects were requested not to relax during an entire period.

During similar occupations and under quiet conditions to favor relaxation, tests were made with 19 untrained normal subjects, but with no request to relax. A diminution of the jerk generally took place. This failed to occur in 3 out of 5 cases with neurotic subjects. Evidence is given that the observed diminution of jerk both in trained and untrained subjects was due chiefly to relaxation of skeletal muscles and not to fatigue, slipping of the patellar tendon or other conditions.

*The effect of ultraviolet light upon thyroparathyroidectomized rats.* FREDERIC T. JUNG.

Thyroparathyroidectomized male white rats, kept on a standard diet and weighed daily, were divided into two groups of equal average weight and were exposed daily to the light from mercury-vapor quartz lamps of known strength. The backs of the rats were shaved, and the dosage was approximately fifty times that necessary to produce an erythema in the unhabituated human subject. One group (controls) were shielded from the ultraviolet component of the light by being covered with glass plates. In the first experiment 26 rats were used, and irradiations were begun on the day of the operations. At the end of an arbitrary time limit the mortality, severity of tetany, and loss in weight were studied; the results coincided in showing a favorable effect of the ultraviolet light. In a second experiment thirteen rats were used, and the irradiations were begun three days before operation. The results were again positive. The conclusions of Swingle and Rhingold from experiments on dogs are therefore corroborated.

*The measurement of muscular tension and its bearing on plurisegmental innervation.* E. KARRER and H. C. STEVENS.

The distribution of the constituent, effector, fibers of a nerve to the muscle fibers of a striated muscle may be conceived of in several ways:

*a*, one nerve fiber to one muscle fiber; *b*, branches of the same axone to several muscle fibers; *c*, double innervation in which two different axones innervate the same muscle fiber. Experiments made to determine which of these possibilities is the true one may be classified as anatomical and physiological.

Physiological observations depend upon the measurement of *a* muscular tension; *b* action current of muscle; *c* heat developed during muscle contraction. The physiological method requires a muscle-nerve preparation in which the same muscle is innervated by two nerve sources, such as the $L_8$ and $L_9$ of the frog which unite to form the sciatic nerve, and the gastrocnemius muscle. The muscle response is measured when $L_8$ is excited alone: then $L_9$ alone and finally by both $L_8$ and $L_9$ together. The problem may be stated by using letters to stand for the nerves and the muscular response. Let *a* stand for one nerve; let *b* stand for the other nerve; let $T_a$ stand for the tension developed when *a* is excited alone; let $T_b$ stand for the tension developed when *b* is excited alone; let $T_{(a+b)}$ stand for the tension developed when *a* and *b* are excited together. The problem to be solved by experiment is whether $T_a + T_b \gtreqless T_{(a+b)}$. The results of physiological investigations show that measurements of heat and action current are additive. Resorting to symbols and letting *H* stand for heat (or action current)

$$H_a + H_b = H_{(a+b)}$$

Most of the reported measurements of tension are not additive. $T_{(a+b)}$ is from 15 per cent to 50 per cent less than $T_a + T_b$. Hence some investigators, relying on these observations have concluded that a certain percentage of the muscle fibers are doubly innervated by axones from both *a* and *b*. Their conclusion is based upon the assumption that a muscle fiber would exert the same force if it were excited by two nerve impulses as it would if excited by one. Analysis of the mechanical conditions under which muscular tension is measured has led us to believe that certain errors in technique tend to make $T_{(a+b)}$ small as compared with $T_a + T_b$ By eliminating these errors we find that Tension is ninety-six per cent additive. The lateral head of the gastrocnemius of decerebrate cats was used in these experiments. An apparatus so constructed as to minimize errors in the measurement of tension is described.

*The influence of varying amounts of carbohydrates on weight curves of two hogs in undernutrition.* ROBERT W. KEETON and HELEN MACKENZIE.

In the present study it was assumed that the hog presents a type of simple or exogenous obesity. The object of the experiment was to determine whether the rate of weight loss in the hog would be different, when he was fed a diet rich in carbohydrates, than when he was fed a diet of the same caloric value but free of carbohydrates. The protein in the diets remained constant, the changes being made entirely in the fat and carbohydrate.

Two male hogs, age 10 months, of the same litter, fed on skimmed milk, corn, and kept in a pasture were selected for the experiment. At the beginning hog 1 weighed 123 kilos and was approximately 159 cm. long and about 84 cm. tall; hog 2 weighed 109 kilos, length 148 cm., and height 80 cm. These hogs were placed on a balanced ration of corn, oats, wheat

middlings, linseed oil meal, alfalfa hay and salt. They were confined to metabolism cages throughout the experiment. The basal requirements were figured by Krogh's figures of 19.1 calories per kilo. The maintenance level for weight was fixed at 16 per cent above this basal level. The maintenance diet computed from the above ration yielded a protein of 0.94 gram per kilo body weight which seemed to be adequate and which was kept constant throughout the experimental periods.

The animals were kept on the basal plus 16 per cent level until the weight remained constant, and then they were reduced to a basal minus 20 per cent level. One animal was placed on a high carbohydrate and the other on a carbohydrate free diet. The experiment was followed by a recovery period and then a second period of undernutrition was established, in which the diets of the animals were reversed. The experimental periods averaged 30 days and the recovery periods 50 days. There were four periods of undernutrition. The experiments were begun July 20, 1926 and terminated February 6, 1928. Every precaution was taken to keep the animals in good condition and free from any deficiency disease.

Results: 1. The hogs lost weight at approximately the same rate on the high carbohydrate diet and on the diet free of carbohydrate.

2. The nitrogen equilibrium was maintained during the greater part of the experimental period when the level of undernutrition was 20 per cent below basal.

3. On both diets the titratable acidity of the urine tended towards the zero point and in one experiment the urine became distinctly alkaline.

4. The ammonia nitrogen increased slightly and equally on each diet.

5. The carbon dioxide combining power at the close of a period of undernutrition corresponding to a level 30 per cent below basal showed values of 70 and 72 on the two diets respectively.

*A comparative study of the criteria of human diastolic blood pressure.* C. E. KING and W. E. GARREY.

Simultaneous graphic records were made on smoked paper of pressure within the arm cuff, of oscillations by the Erlanger method, of volume changes of the forearm, and of the amplified sounds of Korotkow.

All systolic criteria agree sharply, regardless of variations in manipulative procedure. Such is not the case with the diastolic criteria. The speed of inflation of the cuff, and the rate and method of decompression are important factors in determining at what pressure points the characteristic arm volume and accepted sound changes occur. On basis of plethysmographic and auscultatory methods, the slower the decompression the higher will be the diastolic reading. There is less variation in the oscillatory criterion recently adopted by Erlanger.

The sounds of Korotkow depend, in part, on the degree of fullness of the vascular bed below the compression cuff. Absolute diastolic readings by the auscultatory method are practically impossible. In order to make such readings, even of relative value, both compression and decompression must be carried out rapidly and uniformly.

*The effect of evisceration on the respiratory quotient in decapitate cats.* L. G. KILBORN.

The respiratory quotient in the air collected during 15 minute periods

from decapitate cats has an average R.Q. of a little over 0.7, which changes to one of nearly unity or over it after evisceration. This confirms the work of Burn and Dale. It has been found, however, that the rise cannot be taken to indicate the exclusive combustion of carbohydrate in the muscles since it is due to a blowing off of $CO_2$ dependent on the facts that the lungs are being over ventilated and that lactic acid accumulates. By reducing the stroke of the pump after evisceration the R.Q. returns to its original level and the same result was achieved by causing the animal to breathe an atmosphere containing $CO_2$. Examination of the alkaline reserve of the arterial blood shows a marked fall to accompany the rise in quotient following evisceration and the reserve can also be restored to its original level by restricting the breathing of the animal. These results, therefore, confirm and amplify those obtained by Murlin, Edelmann and Kramer.

*The cause of cardiac slowing accompanying postural apnea in the duck.* THEODORE KOPPÁNYI and M. S. DOOLEY.

There is a slowing of the heart during postural and submergence apnea, reaching its maximum after fifteen seconds. The blood pressure may rise during postural apnea, the rise sometimes amounting to more than 100 per cent. This blood pressure elevation is not infrequently suppressed by powerful vagal inhibitions. Section of the vagi or atropinization abolishes the vagal inhibitions during postural apnea and permits the early appearance of blood pressure rises. That the cardiac inhibition and blood pressure elevation are due to insufficient ventilation and increased $CO_2$ content of the blood is supported by the following experiments: *a.* In the latest stages of the apnea, which follows acapnia, there is a slowing of the heart.

*b.* The apneae, which follow the Hering-Breuer stimuli, the stimulation of the glottis, the stimulation of the central end of the divided vagus nerve, the inhalation of ammonia fumes, are characterized by corresponding slowing of the heart and by a more or less definite rise in blood pressure.

*c.* During respiratory pauses, which follow the postural apnea of morphine-urethanized duck and the prolonged occlusion of the trachea, there is a marked slowing of the heart.

*d.* Occlusion of the trachea itself is followed by gradual decrease in heart rate, which quickens at once when the animal is allowed to respire.

*e.* Continuous insufflation of the animal during postural apnea prevents the appearance of cardiac inhibition and blood pressure elevation, or when these are already in evidence, it speeds up the heart rate again and lowers the blood pressure almost immediately.

*f.* Administration of $CO_2$ produces a slowing of the heart.

Premature ventricular contractions may frequently be observed during postural apnea.

*The failure of vaginal smears as an index of oestrus in rabbits.* M. M. KUNDE and T. PROUD.

Daily vaginal smears were made of rabbits of known laboratory stock. No definite cellular content of the lumen of the vagina can be detected which is characteristic of the time when copulation occurs.

*The prevention of fatal parathyroid tetany in thyroparathyroidectomized dogs, by the administration of thyroid hormone.* M. M. KUNDE, R. OSLUND and R. KERN.

Dogs in a hyperthyroid condition may be thyroparathyroidectomized and fed a diet containing 200 to 400 grams of meat daily without the occurrence of fatal parathyroid tetany, provided the hyperthyroid condition is maintained.

The blood calcium and phosphorus are following in these dogs; also the renal function.

*Micro-injection studies of capillary pressure in mammalian mesentery.* EUGENE M. LANDIS.

The gradient of fall in mean blood pressure through the peripheral vessels of the rat and guinea pig mesentery was determined directly by the micro-injection method previously described (This JOURNAL, 1926, lxxv, 548). As observed earlier in frog's mesentery the fall in pressure was not limited to the arterioles but continued also through the capillary vessels. In decerebrate rats and anesthetized guinea pigs the average arterial pressure was approximately 100 cm. of water, and the arteriolar pressure ranged from 50 to 75 cm. In the arteriolar end of the capillary network the average pressure was between 30 and 40 cm., while in the venous capillaries it was reduced to an average of 17 cm. The average venous pressure was 11.5 cm. The osmotic pressure of the plasma proteins as determined for the rat and guinea pig varied between 22.0 and 27.5 cm. water, which was lower than the arteriolar capillary pressure and higher than the venous capillary pressure. It appears therefore that in the mammal, as in the frog, there is a balance between the osmotic pressure of the plasma proteins and capillary pressure, and that the filtration and absorption of fluid through the capillary wall depends primarily upon the relation between these two factors.

*Tissue changes in parathyroid tetany and in guanidine poisoning.* E. LARSON AND LEO A. ELKOURIE.

A series of dogs, cats and rabbits was injected subcutaneously with fatal doses of guanidine hydrochloride. Three dogs were thyroparathyroidectomized and allowed to die in an acute attack of tetany. Both of these series of animals were autopsied immediately and tissues saved for microscopic examination. Any gross changes were noted.

The tissues of the animals dying of guanidine poisoning showed the following changes. The kidneys presented a condition of simple necrosis of the tubules with the glomeruli little affected and no congestion. The liver, in every case, exhibited fatty degeneration at the periphery of the lobules and simple necrosis at the center. The spleens showed no striking changes. The adrenals were in varying stages of simple necrosis. The testes and epididymides appeared normal. Examination of these tissues from the dogs dying of parathyroid tetany revealed the following changes. Both the glomeruli and the tubules of the kidneys were greatly congested with necrosis of the cells of the tubules. The livers were congested to a great degree. Simple necrosis of the hepatic cells was in evidence, however in one case the hepatic cells were apparently little affected. The spleens, in every instance, were immensely congested with the presence of

blood pigment. The adrenals were likewise congested. The various areas presented varying degrees of necrosis.

There were marked differences between the microscopic findings in the viscera of animals dying of parathyroid tetany and those dying of guanidine poisoning.

*The comparative effects on the red-blood cell count of the administration of "spleenmarrow," liver extract and the liver diet in normal humans.* CHAUNCEY D. LEAKE.

Preliminary studies have been made in four healthy adults, to whom "liver extract 343," "spleenmarrow," and a liver diet were administered in varying sequences and with a two weeks' rest period between. "Spleenmarrow" was not administered for longer than four days, but the liver preparations were given from seven to ten days. The latter had no apparent effect in the recommended dosages on the number of circulating erythrocytes in these subjects, whereas 1 to 2 grams daily of "spleenmarrow," as has been repeatedly noted before, raised the red cell count from 600,000 to 1,400,000 above the normal level within three days.

There is apparently a difference between red-blood cell production under normal conditions, or in mild secondary anemias, and in severe secondary anemias or pernicious anemia. Liver is brilliantly successful in treating the latter conditions, but usually has been disappointing in secondary anemias. On the other hand, "spleenmarrow" is clinically helpful in certain persistent mild secondary anemias, but has not been useful in pernicious anemia. There is little justification for the current popular opinion that liver may be used as a means of preventing anemia, or of improving the red cell count in normal individuals or in mild secondary anemias.

*The effects of certain anesthetics (isoamylethyl barbituric acid and ethyl carbamate) on basal metabolism in the rat.* MILTON O. LEE.

The effects on basal metabolic rate of two anesthetics, isoamylethyl barbituric acid (amytal) and urethane, were determined in seventeen adult rats. A modification of the gravimetric method of Haldane, and a modification of the oxygen consumption method of Benedict were used. Amytal was administered subcutaneously or intraperitoneally in dosage of 2.5 mgm., 5 mgm. and 7.5 mgm. per 100 grams body weight. With the smallest dose the metabolic rate was decreased less than 5 per cent. With the dosage 5 mgm. per 100 grams the basal metabolism was decreased an average of 8 per cent, and with the dosage 7.5 mgm. per 100 grams the metabolic rate was decreased an average of 20 per cent. In all cases in which the metabolic rate was followed after recovery from the anesthetic, a return to the original level or a slight increase over it was noted. No significant effect on the respiratory quotient was demonstrated, and no difference between subcutaneous and intraperitoneal administration was found. Urethane in dosages of 60, 75 and 90 mgm. per 100 grams body weight was given to ten animals. The basal metabolic rate was lowered less than 5 per cent in two cases and increased less than 10 per cent in eight. No difference under the three dosages was found, although surgical anesthesia for periods of a half-hour to two hours was produced. No change in respiratory quotient was found.

*Effects of temperature on the development of an insect.* DANIEL LUDWIG.

Different stages of the life cycle of the Japanese beetle (*Popillia japonica Newm*) do not react in the same manner to temperature. The larval stages are modified by other factors such as food and a "resting stage" in development. The position of this "resting stage" is determined by temperature, occurring in the first instar at relatively low temperatures and in the second or third instar at higher temperatures. As a result, there is a negative correlation between the lengths of the different larval instars so that if one is short another is correspondingly long.

The $Q_{10}$ and u values are not constant for any stage but diminish regularly as the temperature is increased, except in the third instar, which is modified by the behavior of the other larval stages.

Krogh's formula, that the rate of development is increased in direct proportion to the increase in temperature, applies to the egg and pupal stages. The number of day degrees required for the complete life cycle is not constant for different temperatures but is larger at higher temperatures.

The effects of alternating as compared with constant temperatures representing their mean values, depend upon the temperatures involved. *a.* If one of the temperatures is above the optimum temperature, development seems to be retarded. *b.* If both are between the threshold and the optimum, neither a retardation nor an acceleration is produced. *c.* If one is below the threshold, an acceleration of development is produced.

*The effect of purified secretin on bile flow from the liver.* H. C. LUETH and GRANT KLOSTER.

Since it has been shown[1] that impure secretin with vasodilatin present causes an increase in bile flow, we thought it advisable to determine the effect of our highly purified vaso-dilatin free secretin on bile flow.

The pancreatic and common bile ducts were cannulated, the cystic duct was clamped above the opening of the hepatic duct from the right lower lobe, and the carotid was connected with a manometer for a blood pressure record, light barbital-ether anesthesia being used. A record of the continuous flow of bile and pancreatic juice was made and then highly purified secretin, active in one milligram amounts was injected intravenously. It was found that the pancreas responded copiously within from one to three minutes and that the bile flow was definitely augmented after a latent period of from seven to nine minutes, no change in blood pressure occurring. The augmentation in bile flow has never been more than approximately ten per cent of the augmentation in pancreatic-juice flow.

*Effect of lutein injections on oestrous cycle of the guinea pig.* DAVID I. MACHT, ARTHUR STICKEL and DANIEL SECKINGER.

The oestrous cycle in guinea pigs was studied over a period of several years by the vaginal smear method of Papanicolaou, Stockard and others. Water-soluble extracts of corpus luteum were obtained by two methods. According to one method freshly desiccated corpus luteum was extracted with petroleum ether, then with pure ether and again with pure ethyl acetate, evaporated to dryness, the residue taken up in aqueous bicarbonate solution and finally neutralized with hydrochloric acid. In the second

[1] Delezene and Frocin. Compt. Rend. Soc. Biol., lvi, 1904.
Bottazzi and Gubrieli. Arch. Internat de Physiol., iii, 1905–1906.

method the corpus luteum was extracted with weak alcohol deproteinized with subacetate of lead, freed from excess of lead with sulfuric acid, evaporated on the water bath to remove alcohol, and again neutralized. Corpus luteum was obtained in the fresh state from cows in some experiments and from the pig in others. Control experiments were made with injections of follicin and extracts of ovarian residue, placenta and various glands. Daily injections intraperitoneally of 1 cc. of the aqueous lutein solutions (equivalent to 5 mgm. of the residue) produced a distinct inhibition of the oestrous cycle which was suppressed in some animals for as long as seventy or more days as compared with the average oestrous cycle which lasts usually from ten to fourteen days. When the injections were discontinued the normal cycle was quickly reëstablished. In the present investigation only perfectly healthy animals were used as it was found that when the animals were diseased the oestrous cycle became very irregular.

*Influence of heparin injections on anaphylactic shock.* DAVID I. MACHT, FITZGERALD DUNNING and ARTHUR STICKEL.

Heparin was obtained by Howell's original method and later by a new method developed in this chemical laboratory which yielded a highly purified and potent product, as indicated by its powerful anticoagulant properties on the one hand and freedom from impurities producing a depression on intravenous injection on the other hand. Three kinds of animals were sensitized by repeated injections of horse serum. The animals used were guinea pigs, rabbits and rats. After the preliminary sensitization the animals were allowed a period of rest of from ten to fourteen days when they were ready for experimentation. In control experiments such animals receiving an intravenous injection of the antigen gave marked evidence of anaphylactic shock. When the sensitized animals were first given an injection of heparin into the blood stream in sufficient doses to prevent the clotting of the blood a subsequent injection of the antigen within twenty minutes after administration of heparin did not produce any anaphylcatic shock at all or in a few animals was followed by only very mild manifestations of anaphylaxis. A summary of all of the results obtained yields the conclusion that heparin tends to prevent serum anaphylaxis when administered in sufficient quantities and shortly before injections of the antigen.

*The formation of ammonia following complete removal of the liver.* FRANK C. MANN and JESSE L. BOLLMAN.

The cessation of the formation of urea in dogs following complete removal of the liver provides a means of studying the formation of ammonia. Following hepatectomy the urea in the blood and tissues decreases progressively, and at the same time its excretion in the urine decreases markedly. As the body becomes depleted of preformed urea, its concentration in the urine becomes extremely low. The ammonia excreted after hepatectomy is equivalent to that excreted by similarly treated normal animals, and it would appear that the amount is related to the acid-base equilibrium of the animals. However, when the urine of the dehepatized dog becomes extremely low in urea, it is always found that the ammonia excretion has likewise diminished. Intravenous injection of urea at this time causes a definite increase in the excretion of ammonia.

The injection of amino acids does not increase the ammonia content of the blood, tissues, or urine of the dehepatized dog, even at a time when the injection of urea would increase the excretion of ammonia. Ammonia or ammonium salts injected into the dehepatized dog may be quantitatively recovered as ammonia in the blood, tissues, and urine several hours afterward. Since the normal animal rapidly converts ammonia to urea, it is evident that this process is not accomplished in the absence of the liver. It would appear that the liver is necessary for deaminization of amino acids and for the formation of urea, but that the liver is not concerned with the formation of ammonia from urea.

*Structure and function of kidney in Lophius piscatorius.* E. K. MARSHALL, JR. and ALLAN L. GRAFFLIN.

The kidney of the goose fish (Lophius piscatorius) has been carefully studied morphologically and functionally. Serial sections of a complete kidney showed only 78 structures which might be regarded as glomeruli. Certain peculiarities of these structures and the fact that no connection could be demonstrated with a tubule make it doubtful as to whether they are true functional glomeruli. A maceration study indicated that all the tubules observed ended blindly. Moreover, an estimate of the number of tubules in the serially sectioned kidney gave 150,000 as the minimum or about 1 pseudo-glomerulus to each 2,000 tubules.

A study of the concentration of various substances in the blood plasma and urine showed only a trace of urea in either blood or urine, a high creatine and low creatinine content of the urine, and marked concentration of magnesium and sulphate by the kidney. After injection of phenol red, it may be 28 times as concentrated in the urine as in the plasma.

The morphological and functional results indicate that in this kidney we have pure tubular excretion of all substances present in the urine.

*Determination of cardiac output in man.* E. K. MARSHALL, JR. and ARTHUR GROLLMAN.

A study was made of the principles underlying the various methods applicable to the determination of the cardiac output in man. As a result of these investigations the use of a foreign gas was found to offer a method which is both accurate and extremely simple in its technique. This method consists in rebreathing from a rubber bag a mixture of ethylene, oxygen, and nitrogen or one of nitrous oxide, oxygen and nitrogen. When complete mixture of these gases and the residual air of the lungs has been obtained (15 seconds) a sample of gas is taken and 10 or 12 seconds later a second sample. These are analyzed for ethylene (or nitrous oxide), oxygen, and carbon dioxide, and from the data obtained together with the solubility of ethylene (or nitrous oxide) in blood, the amount of oxygen taken up by a liter of blood in passing through the lungs is calculated. A determination of the oxygen consumption completes the data necessary for calculating the minute volume of the circulation.

Special experiments have proven that complete mixture of the gases in the lung-bag system takes place in 15 seconds, and that equilibrium of the foreign gas with the blood is attained. No difference in the results is obtained if the whole experiment is prolonged for ten seconds, which indicates that no appreciable amount of the foreign gas is being

returned by the mixed venous blood and that the oxygen content of the mixed venous blood is unchanged from the normal. A series of determinations of the blood flow of various individuals using low concentrations of ethylene (1.5 per cent), high concentration of ethylene (15 per cent), and nitrous oxide (12 per cent), respectively, gave identical results.

The minute volume of the circulation determined in the basal condition varied from 3 to 5 liters in a series of 12 individuals.

*Oxygen consumption compared with excess blood lactates after various anoxemic states.* E. G. MARTIN, JOHN FIELD II and V. E. HALL.

We have previously shown[1] that artificially induced exercise in dogs anesthetized with amytal results in marked increase in blood lactates. Immediately after the end of a fifteen minute period of such exercise the blood lactates average about 130 per cent above the pre-exercise level in our experiments. The rate of oxygen consumption at this time exceeds the pre-exercise rate by about 40 per cent. During the succeeding hour the rate of oxygen consumption (corrected for changes in body temperature) declines slowly, being still about 25 per cent above the pre-exercise level at the end of the time; the concentration of blood lactates also declines, but more rapidly, being about 30 per cent above the pre-exercise level at the end of an hour.

In dogs anesthetized as above, and given curare intravenously until breathing movements are markedly reduced, blood lactates rise as the result of administering gas mixtures in which the oxygen concentration is low (artificial respiration is maintained and $CO_2$ is absorbed out). The extent of rise after an hour of such treatment is comparable to that seen as the result of fifteen minutes of artificially induced exercise. The after decline is also at about the same rate. Following the anoxemia period abundant oxygen is administered, with adequate lung ventilation. The oxygen consumption during the early part of this recovery period is approximately the same as during the initial control period. The rate remains nearly constant as the experiment is continued, although the blood lactates decline markedly, as pointed out above.

In dogs anesthetized as above and in which the portal viscera and liver have been excluded by clamping the celiac axis, the anterior mesenteric artery, the posterior mesenteric artery and the portal vein, the blood lactates are found in our experiments to average only about two-thirds the usual concentration. The oxygen consumption declines, as the result of excluding the viscera, in about the same proportion. Fifteen minutes of artificially induced exercise result in increase of blood lactates to higher concentrations than are obtained by either of the methods described above. The after decline is also more rapid and more extensive. The oxygen consumption immediately after exercise exceeds the pre-exercise rate by about 35 per cent. The rate falls off rapidly, however, reaching the pre-exercise level in about a half hour. The total excess oxygen consumption is markedly less than in the entire animal, exercised similarly, although both lactate production and the subsequent decline in lactates are more pronounced.

*Ultra-violet wave lengths responsible for the cure of rickets in chickens.* GEORGE H. MAUGHAN.

[1] Martin, Field and Hall. 1928, Proc. Soc. Exper. Biol. and Med., xxv, 273.

A study of the curative value of different wave lengths of ultra-violet was made in the case of rickets in chickens. Ninety-four rachitic chickens were divided into ten groups and given exposures to different bands of ultra-violet rays from a quartz mercury vapor lamp by interposing filters of Corex glass.

Table 1 gives the irradiation received by the respective groups for wave lengths between 2755 Å and 3342 Å inclusive. It also gives the per cent recovery for these groups after twenty-eight days' treatment.

TABLE 1

*Total transmission of filters*

In per cent, based on unit exposure of 1 minute (open arc)

| | WAVE LENGTH | | | | | | | | EXTENT OF RECOVERY |
|---|---|---|---|---|---|---|---|---|---|
| | 2755 Å | 2804 Å | 2894 Å | 2926 Å | 2968 Å | 3024 Å | 3126 Å | 3342 Å | |
| | | | | | | | | | *per cent* |
| Open 60 s. (no filter) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 95 |
| Filter A | | | 20 | 33 | 59 | 100 | 170 | 246 | 78 |
| Open 30 s. (no filter) | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 67 |
| Filter B | | | | 9 | 32 | 105 | 370 | 940 | 48 |
| Filter C | | | | | 12 | 50 | 292 | 1,170 | 8 |
| Filter D | | | | | | | | 346 | 0 |
| Open 10 m. (no filter) | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 100 |

Estimates of the amount of recovery were based upon 1, general appearance; 2, growth; 3, post-mortem examinations; 4, x-ray photographs; 5, bone ash, and 6, Ca and P in the blood serum.

Wave lengths 3130 Å and those longer showed no power to cure rickets.

Order of recovery corresponds to the amount of irradiation received from wave length 2968, by the different groups. It was concluded that this is the region of the spectrum most important in curing rickets. While wave length 3024 Å has double the intensity of wave length 2968 its power to cure rickets seemed not more than one-fourth as great as the shorter rays. Wave lengths shorter than 2894 seemed to have little antirachitic power. This is perhaps due to the absorption of the shorter rays by the dead cell layer on the surface of the skin.

*Some phases of the treatment of rickets in chickens by ultra-violet radiations.* GEORGE H. MAUGHAN.

In May, 1926, experiments were begun on the prevention and cure of rickets by ultra-violet irradiations. Chickens were chosen as the experimental animals. They develop rickets, in the absence of ultra-violet radiations or vitamin D, on a balanced diet containing all the ordinary carbohydrates, fats, proteins, and ash materials necessary for normal growth. Under such conditions practically every chicken will develop the disease before six weeks of age, depending upon the food and care of the mother stock.

Five series of tests were run in 1926. They contained 17 groups or a total of 198 chickens. Another series in 1927 contained 12 groups or a total of 142 chickens.

Rachitic chickens seldom, if ever, die of rickets. Out of 177 which had severe rickets averaging more than four weeks five only died, three of rhinitis and two of unknown causes.

Tests were made on the area of the body responsible for the absorption of the rays. The eyes were found not to absorb enough to be of much benefit to the animal in the cure or prevention of rickets. Neither were the eyes affected in a harmful way by close range radiations ($22\frac{1}{2}$ inches from floor of the pen) with periods up to ten minutes' daily irradiation. The regions of the body covered by feathers receive very little or perhaps none of the wave lengths concerned in prevention of rickets.

Cloth similar to that worn by people in the summer prevented ultraviolet from reaching the body. Irradiated air did not prevent or cure rickets. In the cure of rickets the presence of irradiated chickens in the pens with non-irradiated rachitic ones did not benefit the latter; however, when non-irradiated chickens were kept in the pen with irradiated ones, in which rickets was prevented by daily treatment, from hatching time, the untreated animals developed only mild rickets. The controls were all very rachitic at five weeks.

Five minutes' daily exposure to a Cooper Hewitt A. C. Uviarc Poultry Treater at 30 inches from the floor, prevented rickets as completely as did 15 minutes' exposure. Seven minutes' daily treatment cured rickets in four weeks. One minute daily irradiation at a distance of $22\frac{1}{2}$ inches with a voltage of 80 across a Uviarc D. C. burner caused an average of 95 per cent recovery in twenty-eight days. After the first week the growth of this flock was almost as fast as the growth of chickens receiving 10 minutes' daily treatment. Deformities which are the result of long continued rickets are not entirely outgrown. This is most noticeable especially in the spinal column and particularly in the region of the sacrum. Such animals also fail to reach normal size although from all outward appearances they seem perfectly normal.

*Blood changes following carbon arc irradiation.* H. S. Mayerson.

The primary result of abdominal irradiation is a temporary increase in plasma volume of 6 to 37 per cent, the extent and duration of the increase being dependent on the dosage and interval between successive exposures. The blood dilution usually reaches a maximum in each animal after the first exposure which is not exceeded by subsequent irradiation. After massive exposures a slight concentration follows the initial dilution. Repeated exposures in five experiments stimulated the hematopoietic system as shown by increases in red cell number and red cell volume of from 10 to 19 per cent, maintained for from 3 to 6 weeks after the last irradiation. The increase in Hb is proportionately smaller than that of the reds, in two cases there being actual decreases of 7 and 11 per cent respectively in the post-irradiation period. The color, volume and saturation indices indicate that the cells in the post-irradiation period are usually smaller and less saturated than before the irradiation. In two experiments there were post-irradiation rises in platelets of 4 and 15 per cent respectively, persisting for about 10 days. The number of whites fluctuates during and after the irradiation periods but in the majority of dogs ultimately decreases to lower levels, the level in one animal being only 50 per cent normal 4 weeks after the last exposure. Irradiation is generally immediately fol-

lowed by a lymphocytosis, especially marked after massive exposures. With the progressive leucopenia, the lymphocytes usually decrease but not as much so as the polymorphs, thus giving rise to a relative lymphocytosis, maintained for 1 to 4 weeks after the last exposure.

Similar observations on hemorrhagic anemia are now in progress.

*Newly discovered controls of ciliary activity.* JAMES F. MCDONALD, C. E. LEISURE and E. E. LENNEMAN.

Ciliated epithelium, like motile viscera generally, has long been known to possess the property of automaticity, but unlike the latter, has been held to be free from control by the regulatory mechanisms that coördinate the rate of their activity with the functional needs of the body as a whole.

This report shows that the rate of the movement of ciliated epithelium of the vertebrate (mouth and pharynx of frog) is accelerated markedly by stimulation of the sympathetic trunk in that region, and is markedly inhibited by the stimulation of the parasympathetic nerve supply.

Sympatho- and parasympatho-mimetic compounds also give corresponding effects, epinephrine and ephedrine accelerating, and pilocarpine inhibiting this movement. Atropine gives mixed effects, hastening the cilia but by drying the secretions retarding propulsion.

Moreover, ciliary activity is highly dependent upon the presence of moisture. Drying by means of gentle air draughts slows or entirely inhibits this activity, depending upon the degree of moisture removal. Motion returns after partial drying, but not after the drying has been complete.

We are now studying by similar methods the ciliary mechanism of mammals, with the view not only of clarifying our understanding of the foundational mechanisms which serve to explain the coördination of ciliary activity with the physiological needs of the higher organism as a biologic unit, but also of explaining what should be an important factor in the mechanism underlying malfunction of systems, as respiratory and genito-urinary, which contain ciliated epithelium as a component tissue. Dehydration paralysis of cilia should for example prove to be an important etiological factor in relation to respiratory infections, widely prevalent during cold seasons when the atmosphere of homes, offices, etc., is known to be so dry.

*Lactic acid metabolism of the circulated brain.* DANIEL A. MCGINTY.

Simultaneous samples of arterial blood from the carotid artery and venous blood from the superior sagittal sinus were taken during anoxemia by cyanide injection or by clamping the carotid and vertebral arteries. Normally the lactic acid content of sinus blood is somewhat below that of arterial blood. Immediately after injection of cyanide the lactic acid of sinus blood approaches or equals that of arterial blood but rapidly falls below the arterial level. After the first effects of cyanide have disappeared the lactic acid of sinus blood again approaches and frequently exceeds the arterial level to fall again below the arterial. At this time the excessive lactic acid production by brain tissue is evident in the blood stream. During the course of the experiments both sinus and arterial lactic acid were increased and both were always below the lactic acid level of venous blood from a circulated leg. The results of anoxemia by clamping the arteries to the head were similar.

*The starting time of athletes measured in group competition.* WALTER R. MILES.

A multiple chronometer for use on the athletic field has been devised which makes possible the measurement of seven men at once. The method of recording is by releasing golf balls which fall and strike a revolving drum. The men can see the results immediately after making a trial. In studying the football charge it was found that 86 players, tried seven charges each, gave an average starting time of 0.389 second. The correlation between body weight and speed of charge was found to be $-0.22 \pm 0.09$ for a group of 53 players, thus the heavier men were slower. Men playing the positions of back, end, and guard gave the averages 0.360, 0.377 and 0.383 second respectively. The method is illustrated by motion pictures.

*Guanidine as a factor in the production of intoxication following liver injury.* ANN S. MINOT and JESSIE T. CUTLER.

The protective action of calcium against signs of intoxication following the administration of carbon tetrachloride to dogs has previously been reported. When calcium is deficient death appears to be due to hypoglycemia or tetany and frequently to a combination of the two conditions. A similar picture of intoxication can be produced in a normal dog by the administration of guanidine hydrochloride. Following liver injury, due to carbon tetrachloride, an increased concentration of guanidine compounds in the blood has been demonstrated. This increase is aggravated by a meat diet and by giving alcohol with carbon tetrachloride. The antagonism between guanidine and calcium is well known. The symptoms elicited by a given concentration of guanidine in the blood are increased if the calcium ion concentration is depressed as is the case in the bilirubinemia following liver injury. The conclusion is reached that guanidine is at least one factor in the production of the symptoms noted following severe liver injury.

*Influence of internal secretions and of trephones on phagocytosis.* SERGIUS MORGULIS and B. A. SCHATZILLO.

Phagocytosis in normal serum as well as in serum of castrated or thyroidectomized animals is increased through the addition of trephones. The addition of trephones to thyroxin increases the phagocytosis of normal serum or of serum from thyroidectomized animals. The phagocytosis of serum from castrated animals is little altered from that of normal. Phagocytosis is diminished both after thyroidectomy or after parathyroidectomy. The phagocytic reaction *in vivo* of parathyroidectomized dogs is lowered. Trephones exert no effect on the placental hormone, and the placental hormone has practically no influence on phagocytosis. Under the influence of trephones not only the number of phagocytosing cells increases but the quantity of material ingested is likewise markedly increased.

*The energy for muscular contraction in starvation as evidenced by ketone body and nitrogen excretion.* A. G. MULDER.

In order to test the possibility of fat burning in muscular contraction during carbohydrate deficiency, the ketone body and nitrogen excretion were measured during starvation. Human subjects were used, and the starvation period was from three to six days. During the latter part of

starvation a period of severe exercise was undergone. In general the ketone body excretion in the urine was decreased rather than increased during the exercise period, while the nitrogen excretion was at the same time slightly decreased, eliminating the possibility of antiketogenic substances coming from protein. Two possibilities present themselves in explaining these results. First, it is possible that there was, even after five days' starvation, sufficient glycogen in the muscle to be burned in the emergency, which burning lowered the ketosis. Or else that the muscle has a different type of ketone body metabolism than the remainder of the body and is able to burn fats completely.

*The influence of extracted liver on blood regeneration.* GULLI LINDH MULLER and EMILIE GOODE.

In a previous study it was found that in pigeons, in which anemia and bone marrow depletion had been produced by starvation, grain and meat feeding caused a rapid and complete regeneration of the blood while broiled liver produced a rapid gain in weight, regeneration of the blood up to a point below normal, followed by a rapid decline of red blood cells and hemoglobin and a definite suppression of the megaloblast formation in the bone marrow. With the same experimental conditions, liver was fed which had been extracted twice with 45 per cent alcohol for 24 and 48 hours respectively and air dried. The animals in most instances regained the weight, red blood cells and hemoglobin in the same way as the controls, and the bone marrow was normal. These experiments on cell growth in vivo lend support to the work by Heaton and others, who found that an inhibitory substance is present in the liver which suppresses cell growth in vitro, and that failure of continued megaloblast formation in pigeons is not due to a deficiency of the liver as a food.

*The absorption of insulin from Thiry Vella fistulae in normal and depancreatized dogs.* JOHN R. MURLIN and RUTH E. LATTA.

The results indicate that fistulae prepared several months before being used for experiments with insulin absorb the hormone better than freshly prepared fistulae. Weak alkalis up to 20th normal favor absorption. Weak HCl up to 100th normal does not retard absorption, but greater strength does in most of the tests.

Blood serum retards the destruction of insulin by pepsin-HCl. This is due to the buffering effect of the serum proteins on the acid. When acid stronger than N/40 is used with ox-blood serum there is destruction of insulin by pepsin. Blood serum alone or serum and HCl up to N/70 does not prevent destruction of insulin by trypsin.

*Rat activity and adrenalin.* L. B. NICE, D. S. GREENBERG and S. L. GREENBERG.

The activity of three groups of rats was studied. A normal group was given 1 cc. of 1/50,000 adrenalin chloride hypodermically daily; a second group of adrenalectomized rats was administered an equal amount of adrenalin in the same way while a third group of adrenalectomized rats was not injected.

The spontaneous activity of the normal rats was decreased on the whole while the activity of the injected adrenalectomized rats closely paralleled that of the non-treated group.

*Experiments with follicular and placental extracts.* R. M. OSLUND.

Feeding of follicular or placental extracts is ineffective. Young female rats were given five unit doses of Estrogen (Parke, Davis & Co.) per day over a period of five days. No oestral changes were found by the vaginal smear method.

Estrogen applied to the vagina in minimal doses was followed by positive oestral changes.

Though follicular and placental extracts when injected subcutaneously or intraperitoneally or when applied to the vagina invoke positive oestral changes, as determined by the vaginal smear method, they do not bring about receptiveness on the part of the female for the male. In three series of spayed rats (18 rats) and in four series of unoperated rats (30 rats) all of which gave positive oestral smears over a period of from one to ten days all females vigorously fought off males four or five times daily. In another series, five rats were spayed and autotransplants made of their ovaries. One month later they were kept in oestrus over a period of nine days. One rat of this series received several males on the seventh day. All others vigorously fought off males four or five times daily.

That the failure to obtain positive copulatory results did not lie in the extract was determined by injecting fresh follicular fluid obtained from hog ovaries. The same results were obtained.

*The reversal of the nerve supply to the stomach of Necturus.* T. L. PATTERSON.

Previous work has demonstrated that the inhibitory influence of the vagi on the stomach of *Necturus* is predominantly, if not exclusively, inhibitory, a phenomenon which is contrary to that found in the stomachs of the higher animals. The method of acute experimentation was continued in which the cervical cord was transected under ether anesthesia, followed by a stomostomy, the dorsal isolation of the vagi and a laparotomy for the purpose of stimulation of the gastric sympathetic fibers. Electrical or mechanical (traction) stimulation of the splanchnic nerves usually calls forth a peristaltic contraction of the stomach which may be inhibited by slight to moderate traction of the peripheral vagus. Simultaneous stimulation of the cut vagus and the splanchnic produces a negative effect, showing that the inhibitory fibers of the vagi predominate over the motor excitatory fibers of the gastric sympathetic in this animal.

*The influence of prostatectomy on the sex life of the male albino rat.* T. L. PATTERSON.

Removal of both the seminal vesicles and the prostate from male rats produces absolute sterility but it has not been determined if sterility follows complete prostatectomy alone. Studies made on young male rats prostatectomized at five to six weeks of age, mated with normal females and observed over a period of twenty months become sterile, but this only occurs when the extirpation of the gland is complete as evidenced by histological studies. The sexual instincts and sexual activity develop in these animals in normal strength, though with some delay, which is probably due to operative interference in a region very sensitive to injury. This indicates that the development of the somatic sex characters and of the psycho-sexual behavior is independent of the presence of the prostate and of any endocrine function which this organ might possess.

*Effect of fat ingestion on ketone body and nitrogen excretion in starvation and the effect of exercise on ketosis during fat feeding.* I. E. PHILLIPS.

In order to find whether fat feeding increases ketosis during starvation and whether it evidences a protein sparing function, fat was fed to starving human subjects. The results in general appear to indicate a very marked increase in ketosis due to fat feeding with apparent protein sparing action.

Exercise during fat feeding does not cause any increase in ketone body or nitrogen excretion. This indicates that even when fat is known to be abundant the metabolism of exercise is not different from that in the starving subject. Calculation shows that the energy for the exercise carried on by the fat fed individual could not have been derived from stored carbohydrate or from the metabolized protein, but must have been quite largely derived from fat.

A typical experiment:

*I.E.P.—I*

| DAY | DIET | CONDITION | KETONE BODIES—24 HOURS | TOTAL N PER 24 HOURS |
|---|---|---|---|---|
| | | | *grams* | *grams* |
| 10 hours | Fast | | | |
| 1 | Fat* | Ord. act.† | 2.160 | 11.923 |
| 2 | Fast | Ord. act. | 10.431 | 17.440 |
| 3 | Fast | Rest | 10.499 | 15.202 |
| 4 | Fast | Exercise | 8.687 | 12.386 |
| 5 | Fast | Rest | 11.663‡ | 12.114‡ |

* Fat consisted only of ¼ pound butter.
† Ordinary activity.
‡ Calculated for 24 hours on a basis of 16-hour urines.

*II*

| DAY | DIET | CONDITION | KETONE BODIES—24 HOURS | TOTAL N PER 24 HOURS |
|---|---|---|---|---|
| | | | *grams* | *grams* |
| 8 hours | Fast | | | |
| 1† | Fast | Ord. act. | 0.583 | 8.61 |
| 2‡ | 200 cc.* | Ord. act. | 15.345 | 14.06 |
| 3‡ | 200 cc.* | Exercise | 14.748 | 12.83 |
| 4 | 200 cc.* | Rest | 19.800 | 9.20 |
| 5‡ | Fast | Ord. act. | 10.382 | 8.71 |

* Pure Italian olive oil.
† Three 10-minute baths at 10° to 15°C.
‡ One 10-minute cold bath at 10° to 12°C.

*A metabolism chamber which automatically maintains a constant partial pressure of oxygen.* H. F. PIERCE.

The chamber consists essentially of two water-sealed spirometers of equal size, the carefully counterbalanced bells of which are rigidly joined together so that they rise and fall as one. To spirometer A are connected the usual absorbing train for water and carbon dioxide, and, by means of a Mueller valve, a gasometer which contains oxygen. To spirometer B is connected a tank having a volume approximately equal to the absorbing system of spirometer A. By putting more or less water into this tank, the

gas volume of system B can be adjusted to equal that of system A. Since there is the same volume of gas in each of the two systems, and the areas of the two spirometer bells are equal, the rigidly joined bells will rise and fall together under the influence of temperature and barometric changes without producing a pressure differential between the systems A and B.

The animal is placed in spirometer A. As oxygen is consumed and the excreted carbon dioxide and water are absorbed, the gas volume of system A becomes less, and a pressure differential is produced between systems A and B, and between system A and the atmosphere. When this differential becomes great enough to overcome the resistance of the Mueller valve, oxygen is drawn into system A from the oxygen gasometer. The oxygen tension is thus automatically maintained approximately constant.

Provision is made for supplying the animal with food and water, and for removing the solid and liquid excreta, without materially changing the oxygen tension in the chamber.

*Some observations on the effects of experimental hemisection of the spinal cord.* F. H. PIKE.

It has been known for many years that an animal may recover a considerable degree of control of the movement of a limb below the level of an experimental hemisection of the spinal cord, but little attempt has been made to analyze the mechanism of this recovery of function. The following observations are submitted on this point.

If the spinal cord is hemisected on the left side in the lower thoracic region, the animals regains sufficient control of the left hind limb to begin walking on it within ten days or two weeks. There is no striking ataxia in the movements of the limb such as one might expect from the interruption of the cerebellar pathways. Some of the symptoms which one might expect to result from interruption of the pyramidal and other descending pathways are absent. The hind limb can not be placed in unusual or uncomfortable attitudes without protest.

If the dorsal roots of the lumbar nerves are divided on one side, say the left, and a period of recovery of some months allowed to elapse, the movements of both hind limbs are lost when the spinal cord is hemisected on the side opposite to the dorsal root lesion. This result was observed in one dog two years and more after section of the dorsal roots. The movements of both hind legs were regained at about the same time, that is, two weeks after the hemisection of the cord.

Intravenous injection of absinthe under local anesthesia is followed by clonic convulsions in three of the limbs, but the hind limb on the side of the hemisection may be outstretched in tonic extension if the injection is made within the first ten days after the hemisection. In one animal in which the hemisection was rather low in the lumbar cord, the Babinski reversal of the plantar reflex in the hind foot on the side of the hemisection was strikingly apparent after a moderate dose of absinthe. The vesical retention, which led to the sacrifice of these two animals so soon after the hemisection, was abolished by the absinthe. Copious and prolonged micturition followed a moderate dose.

Ablation of the left cortical motor area some months after a left hemisection in a cat affected the movements of the left hind limb as well as those of the right. Subsequent lesion of the right lateral lobe of the cerebellum

in the same animal was again followed by an effect on the movements of both hind legs. Lesion of the right cerebellar hemisphere some weeks after a left hemisection of the cord was also followed by an effect on both hind legs.

Lesion of the left cerebellar hemisphere after a section of several, but not all, dorsal roots of the lumbar nerves on the left side was again followed by a transient loss of control of movement in the left hind leg. There was no hemisection in this case.

*Metabolism in pregnant rabbits.* W. T. POMMERENKE and H. F. HANEY.

The object of this series of experiments which is still in progress is to determine to what extent metabolism in rabbits is altered by the state of pregnancy, and to find the effect of thyroidectomy upon metabolism during pregnancy.

A modified Haldane open circuit apparatus was used. The amounts of oxygen used and carbon dioxide given off were determined, and from this data the respiratory quotient calculated. The caloric equivalent of a liter of oxygen at this respiratory quotient was then determined by means of a Du Bois chart.

Five groups of rabbits were used, namely, *a*, normals; *b*, pregnant animals; *c*, thyroidectomized animals; *d*, pregnant animals which were thyroidectomized from 10 to 12 days following impregnation; *e*, thyroidectomized animals which later became pregnant.

Summarizing the results of some 250 tests the following conclusions seem justified. There is a progressive increase in the metabolic rate during pregnancy in rabbits which reaches its maximum during the last five days before parturition. Following parturition there is a gradual fall in metabolism which reaches the normal level in about two weeks. Following thyroidectomy there is a progressive fall in metabolism which reaches its lowest level (about minus 33 per cent) after four weeks. In animals which become pregnant following thyroidectomy there is a tendency to an increased metabolism as a result of their pregnant condition and to a decreased metabolism as a normal consequence of thyroidectomy. The same general state of affairs seems to exist in the group of rabbits which were thyroidectomized 10 to 12 days following impregnation. Thus the metabolism in these latter two groups falls between that of the normal pregnant group and the thyroidectomized non-pregnant group. Following parturition metabolism falls as in case of normals.

*Further report of a case of experimental canine gigantism.* T. J. PUTNAM and E. B. BENEDICT.

A sterile extract of the anterior lobe of beef hypophysis has been administered intraperitoneally to a thoroughbred bull-dog, in doses up to 50 cc. daily, for about a year. The treated animal has outgrown her littermate sister control by 60 to 90 per cent, and shows changes in the bones, skin, nipples and vagina which might be interpreted as acromegaloid. Some of the disabilities of experimental hypopituitarism in dogs can be mitigated by injections of a similar extract.

*The combination of oxygen and hydrochloric acid with the hemocyanin of Limulus.* ALFRED C. REDFIELD and ELEANOR D. MASON.

The influence of hydrogen ion activity upon the equilibrium of hemoglobin and the hemocyanins with oxygen indicates the importance of the combination of acids and bases with these proteins in relation to the oxygen dissociation curve. The hemocyanin of Limulus polyphemus reacts with hydrochloric acid to form a compound which may readily be distinguished by the fact that it is colorless, does not combine with dissociable oxygen, and is insoluble in the presence of electrolytes. Hemocyanin combines about $160 \times 10^{-5}$ mols HCl per gram, and behaves in its total acid combination as though all the basic groups reacted with acid alike and independently of each other. The acid titration curve is approximated by the Henderson-Hasselbalch equation, pK equalling 3.3. In contrast, the change from the colored to the colorless form on the addition of HCl progresses according to the equation,

$$\frac{(\text{Colorless})}{(\text{Colored})} = K(\overset{+}{H})^2$$

It is half complete at pH 3.7 and complete with the addition of about $120 \times 10^{-5}$ mols per gram of hemocyanin. It may be concluded that this reaction involves only a limited portion of the total basic groups, perhaps only those groups in the radicle immediately associated with oxygen transport. The colored fraction appears to dissociate from oxygen according to the mass law. The oxygen dissociation constant does not vary greatly over the range of pH 4.6 to 3.8.

*Interrelations of calcium, phosphorus and sugar in parathyroidectomized dogs.* C. I. REED.

In an effort to explain the nature of the adaptation occurring in latent tetany when animals survive without restoration of normal proportions of calcium and phosphorus it was noted that ingestion of 2 gm. per kilo of glucose frequently relieved dogs from an attack of tetany within 30 minutes to 2 hours. In controlled dextrose tolerance tests it was found that during an attack of tetany there was decreased tolerance which was usually at the peak at the end of 2 hours while in normal dogs and those not in tetany this point was found to occur about the end of the first hour; the fasting blood sugar level was not affected by parathyroidectomy. All fractions of phosphorus were decreased in concentration from 10 per cent to 50 per cent. Calcium was not greatly affected in normal dogs but after operation there was a tendency to fluctuation with ultimate diminution. The action was usually completed in two hours. The diminution in concentration of inorganic phosphorus was usually sufficient to raise the Ca:P ratio to a higher figure and relief from tetany may, at present, be assigned to this factor.

In order to discover what would be the response to decreased blood sugar concentration, insulin was injected subcutaneously. In both normal and operated dogs, with and without tetany, dextrose and inorganic phosphorus concentration were lowered to about the same degree. In tetany the calcium concentration tended to fluctuate while in normal and operated dogs not in tetany this factor was invariably increased. In every case of tetany the net result of insulin injection was an increase of the Ca:P ratio which, if great enough, resulted in relief from tetany.

*Experimental attempts to alter cytoplasmic pH of Amoeba dubia.* PAUL REZNIKOFF and HERBERT POLLACK.

Attempts were made to alter the cytoplasmic pH of Amoeba dubia which was previously found to have a value of $6.9 \pm 0.1$. By the micrurgical technique the various Clark and Lub indicators were injected into the cell and then acids, simple salts like NaCl, KCl, $CaCl_2$, $MgCl_2$, buffer mixtures and $AlCl_3$ were introduced and the effect on the apparent pH noted.

The results showed that in the case of Amoeba dubia not enough acid of injury is produced by the simple insertion of a needle or pipette to register a change in the color of the indicator.

HCl, $CaCl_2$ and $AlCl_3$ produce a marked increase in acidity which, if not buffered immediately, results in death. If localized, the affected region is discarded. $MgCl_2$ also produces acidity and death but no localization occurs. In the case of injection of NaCl and KCl no change in pH of the healthy cell is obtained. Death changes a cell so that it becomes freely permeable to the environment and the apparent pH of such dead cytoplasm is really that of the surrounding medium. If sufficient acid, alkali, or buffer mixture is injected so as to permanently change the cytoplasmic pH by overwhelming its buffering power, then the cell dies.

*The dependence of the electromyogram from voluntary contractions on the anterior horn cells.* CURT P. RICHTER.

The electromyogram from voluntary contractions is greatly altered by lesions of the anterior horn cells found in progressive muscular atrophy, poliomyelitis, and syringomyelia. The frequency of the primary waves may be reduced from the usual 50 to 75 per second to 5 to 10 per second, and each wave stands out clearly, free from Nebenzacken. In some instances two, and occasionally three entirely independent series of primary waves with different frequencies were recorded, just as was found by Adrian and Zottermann in their work on the electroneurogram from proprioceptive end-organs. It was evident from these records that the frequency of the primary waves increases with the intensity of the contraction and decreases with fatigue. Other conditions not involving the anterior horn cells, such as dystrophia myotonica, in which there is a marked atrophy of the muscle, do not show this type of electromyogram.

*Inorganic and organic diet factors in hemoglobin regeneration during simple, severe, secondary anemia in dogs.* F. S. ROBSCHEIT-ROBBINS, C. A. ELDEN and G. H. WHIPPLE.

Previous publications have described the effect of different fruits and organ tissues in blood regeneration in simple severe anemias of long duration in dogs. Peaches and apricots either fresh, canned or dried, when added to a basal bread ration produce considerable regeneration of hemoglobin and red cells in this type of anemia. The observations suggested the possibility of effective inorganic material present in these fruits.

Apricots as well as liver and kidney have been ashed and this material tested as to its potency for hemoglobin regeneration. The results obtained are rather uniform when compared with values obtained from equivalents of fresh substance. The output of hemoglobin following the addition of this inorganic material to the basal bread ration may amount to approximately 40 per cent to 50 per cent of that observed with fresh material.

Even such different materials as fruit and organ tissues may have a common substance or group of substances present in both which may influence this blood picture. Work is in progress with isolated and combined inorganic factors. Various organic substances are being tested as to their potency in stimulating blood regeneration. Actual results obtained will be discussed and illustrated.

*Adrenal insufficiency: The beneficial influence of cortical adrenal extracts in adrenalectomized animals.* J. M. ROGOFF and G. N. STEWART.

Administration of adrenal cortical material prepared by extraction, in various ways and with various solvents, is capable of prolonging life of completely adrenalectomized animals well beyond the maximum period of survival of control, untreated adrenalectomized animals. In animals which do not live beyond the maximum period of survival of adrenalectomized, untreated controls, as well as those which exceed this period, the usual symptoms are generally ameliorated.

*The probability of lactic acid being found in the sweat of normal subjects.* A. K. SAIKI and G. A. TALBERT.

By using the method of Friedman, Cotonio and Shaffer we have gotten lactic acid reaction in 5 determinations which yield from 71 to 160 mgm. per 100 cc. We are taking due precaution, however, as there is a possibility of the presence in sweat of interfering substances which might yield under the conditions very volatile bisulphite-binding products. Pending further investigations we shall only provisionally call the bisulphite-binding substance lactic acid.

*Uric acid in the sweat of normal subjects.* A. K. SAIKI and G. A. TALBERT.

Both the Folin and Brown methods were used for the determinations. While the amount is small yet we discovered it to be constant in 44 experiments on 17 different subjects. We have found it ranging from a minimum of 0.77 to a maximum of 2.52 mgm. per 1000 cc. of sweat.

*Abnormal electrocardiographic tracings due to changes in the ionic concentration of the perfusing fluid.* JANE SANDS and MARGARET SUMWALT.

In an article now in press, one of us is reporting a reversal of the P, R, and T waves of the electrocardiogram caused by shifting the pH of the perfusing fluid. Changes in rate, in conduction, and in direction of some of the waves can also be obtained by causing ionic imbalance when the reaction of the perfusate is kept constantly at about pH 8.0. These abnormal effects are reproducible, are reversible, and in form simulate various abnormal waves commonly observed in clinical electrocardiography.

*The respiratory exchange of man during and after muscular exercise at low barometric pressures.* EDWARD C. SCHNEIDER.

The minute-volume of carbon dioxide expelled during any selected load of work has been approximately the same at all pressures studied, the lowest of which was 290 mm., equivalent to an altitude of 25,000 feet.

While the volume of carbon dioxide eliminated during muscular exercise is unaffected by the reduction in barometric pressure, the consumption of oxygen is decidedly lessened. This is interpreted to mean that an unusual

amount of preformed carbon dioxide is blown off from the blood when work is performed at low atmospheric pressures. This blowing off of carbon dioxide may be due either to an increase in the acid content of the blood or to the effect of anoxemia on the respiratory center. Experimental data support the theory that this equality of output of carbon dioxide during exercise is the direct effect of a lack of oxygen on the respiratory center.

*Pregnancy anemia in rats.* J. M. DUNCAN SCOTT.

The blood volume of pregnant rats is of the same order relative to their *gross* body weight (including fetuses) as that of non-pregnant rats.

The hemoglobin of pregnant rats expressed in grams per gram *net* body weight (excluding fetuses) is diminished as compared with that of non-pregnant rats.

Thus the hypothesis that diminution in hemoglobin percentage during pregnancy in rats is brought about by dilution of the blood is insufficient to account for the facts and we must postulate in addition a diminished formation or an increased destruction of hemoglobin in these animals during pregnancy.

*"Pink-belly disease" in frogs.* J. M. DUNCAN SCOTT.

This ceases to occur when non-chlorinated water is used in the frog-tanks.

*The effect of fatigue and of treatment with certain solutions on heat rigor in excised muscle of frog.* GEORGE DANIEL SHAFER.

1. Confirmation is made (with certain modifications in detail) of the finding of Caroline W. Latimer (1898) that fatigued gastrocnemius muscles of frog entered into heat rigor at a lower temperature and shortened much less than their mates which had not been fatigued.

2. It was found that fatigued, excised gastrocnemius muscles began shortening on the average at a temperature 8.7°C. lower than their non-fatigued mates (other conditions similar) and that they shortened, on the average, 24.3 per cent less than their mates in heat rigor. Fatigued sartorius muscles began rigor at 1.5° to 0.5°C. lower than their non-fatigued mates and shortened, on the average, 40 per cent less than their mates. The comparisons made are for the first step in heat rigor shortening.

3. Completely fatigued excised gastrocnemius muscles, left standing several hours in air (at 18° to 24°C.), were not restored, in their ability to shorten after the manner of their non-fatigued mates, in the least. If completely fatigued gastrocnemius muscles were exposed to pure oxygen (16° to 18°C.) for similar periods, however, a recovery of a little over 50 per cent in ability to shorten was indicated, and rigor began then, on the average, at only 5°C. lower temperature than in case of the unfatigued mates—instead of at 8.7°C. lower temperature. No recovery was indicated in oxygen at 25.5°C.

4. Excised gastrocnemius muscles completely fatigued in pure $CO_2$ shortened, on the average, only 7.4 per cent less than their non-fatigued mates under the same conditions (as compared with 24.3 per cent less shortening for muscles fatigued in air) but they began shortening at a low average temperature of 10.4°C. below that of their mates. Standing in pure $CO_2$ for the period of these experiments did not noticeably change the temperature at which the non-fatigued muscles began heat rigor.

5. Fresh excised sartorius muscles, submerged for a few hours in Ringer's solution made acid (about pH 5) with lactic acid, hydrochloric acid or sodium acid phosphate, until after they had been rendered unable to respond to stimuli, required from 1° to 20°C. *higher* temperature (according to the length of time treated), to start the rigor shortening in air, than did their mates kept during the same time in Ringer's solution at pH 8.4.

6. The temperature at which sartorius muscles began rigor was not influenced by treating the muscles with Ringer's solution containing 1 per cent sodium lactate at pH 9.4.

7. Excised sartorius muscles, submerged in isotonically equivalent alkaline Ringer's solution (pH varying from 9 to 10.8—made alkaline with $Na_2HPO_4$, $NaHCO_3$, NaOH, KOH, $Ca(OH)_2$ or $NH_4OH$) until after they had been made unable to respond to stimuli, began rigor in air at from 1°C. to 8°C. lower temperature, and they shortened less than did their normal mates kept during the same time in Ringer's solution of pH 8.4. That is, the heat rigor phenomena observed in case of fatigued muscles were duplicated by treating muscles for the proper length of time with the properly modified Ringer's solution, and this result could be obtained only with *alkaline* solutions. During longer and longer periods of treatment with alkaline Ringer's solutions, muscles progressively lost ability with respect to the extent to which they could shorten in heat rigor, until they reached a state in which the first step of rigor shortening had disappeared completely.

8. In the case of muscles treated with Ringer's solution made alkaline with $Na_2HPO_4$ it was shown that they were not dead at the time when they ceased to be able to respond to electrical stimuli (or for at least a short time thereafter), because a few minutes' treatment with Ringer's solution containing excess $CaCl_2\left(\frac{m}{32}\right)$ sufficed, yet, to restore them so that they would respond to electric stimulation with definite contraction.

*The effects of selective solar radiation on the parathyroid glands and on growth.* CHARLES SHEARD and GEORGE M. HIGGINS.

A group of two hundred chicks, of the same age and stock, were divided into eight groups and placed under vitaglass, ordinary glass, blue glass (Pittsburgh 56) and amber glass (Pittsburgh 48) both with and without cod-liver oil, respectively, added to the standard (Wisconsin) ration. Hyperplasia of the parathyroid glands developed within three weeks under the blue and amber filters on a diet devoid of cod-liver oil. Under these filters, and with cod-liver oil, the glands are larger than in the chicks under vitaglass or ordinary glass with cod-liver oil. At the end of a month the parathyroids of chicks under amber and blue filters and without cod-liver oil approximate, in size, nine times that of the glands in chicks under vitaglass. Progressive changes within the parathyroids became more and more evident, two distinct types of cystic degeneration occurring.

No marked changes in growth, as evidenced by weight, were found in the chicks under any of the filters during the first three months. At the end of six months' time, the chicks under the blue and amber filters showed 20 and 30 per cent deviations in weight from those reared under vitaglass and on the same diet. These results are in contradistinction to those of other experimenters and will be discussed. The experiments indicate that

normal growth and normal development of the parathyroid glands will be maintained best in the presence of both the lesser and greater wavelengths of sunlight.

*The effect of different amounts of sexual indulgence in the albino rat. VI. Life span and cause of death.* JAMES ROLLIN SLONAKER.

The animals were divided into five groups and the amounts of sexual activity permitted each group was as follows: Group A consisted of pure virgins and strict bachelors which were allowed no intermingling with the opposite sex throughout life. Group B, light breeders, was composed of animals whose sexual activity resulted in not less than two nor more than three litters which were reared. Group C, medium breeders, had as many litters throughout life as they could rear. In group D, heavy breeders, the young were destroyed at birth and the mothers remated as promptly and as often as possible. Group E, excessive coitus, consisted of hysterectomized rats and normal males which were constantly together. Since the removal of the uterus in the rat does not interfere with the normal oestrual cycles, this group had excessive coitus without the ability to bear young. The autopsy records show that the effect on the life span and cause of death was as follows: The average life span of the females of the different groups from the shortest to the longest was E 817 days; A 890 days; D 906 days; C 923 days; B 981 days. A similar order for the males was A 899 days; E 906 days; B and D 975 days; C 1079 days. Normal reproduction was more favorable to longevity of both the female and male than chastity. The virgins had the greatest variation in age at death of any group. The youngest age was 466 days and the oldest 1357 days which was the oldest age recorded for any group. Excessive sexual indulgence without bearing young tended to shorten the life span of both sexes. Destroying the young at birth and promptly remating shortened the life span of the female more than almost continuous normal reproduction during the entire breeding age. Both sexes in which no sexual indulgence occurred had shorter average life spans than the breeding groups. The females were more affected than the males. Diseases of the lungs in the females from greatest to the least was A 53.6 per cent; D 50 per cent; E 45.4 per cent; C 25 per cent; B 22.2 per cent. In the males it was C and D 50 per cent; E 40 per cent; B 25 per cent; A 16.6 per cent. Tumors which appeared late in life were most common in the females of groups C and D. They were much more prevalent in females than in males. Digestive disturbance was common in old animals of both sexes in all groups. In the animals which had no apparent organic trouble and were classed as having died of old age group B of the females and group C of the males showed the highest per cents. Both sexes of group A showed low per cents. Sexual indulgence increased the stature of the female. The order of average body length in millimeters from the least to the greatest was A 181.2; B 183.5; C 184; E 186.5; D 190. The meager data available do not indicate a like effect on the male.

*A study of the action of theophyllin and theobromin on the coronary circulation of the intact heart.* FRED M. SMITH and GEORGE H. MILLER.

Dogs were employed in this series of experiments; a continuous blood pressure record was obtained. The rate of the coronary circulation was

determined by introducing a Morawitz-Zahn cannula into the coronary sinus. The blood volume was maintained by reintroducing the blood at a uniform rate through a cannula in the femoral vein. Heparin was used to prevent coagulation. Solutions of the drugs were injected very slowly into the femoral vein in doses of 5 mgm. per kilo which is comparable to the therapeutic dose for man.

Theophyllin produced an increase in the coronary flow which varied from 50 to 80 per cent. The onset of the action of the drug was indicated by a reduction in blood pressure affecting particularly the diastolic phase. There was a coincident increase in the flow from the coronary sinus which becomes more marked as the blood pressure assumed the original level.

Theobromin was much less effective. In some experiments there was no significant change in the coronary circulation. In other instances an increase flow was observed which varied from 15 to 25 per cent. There was usually some reduction in blood pressure which, however, was never as great as that produced by theophyllin.

*Vago-cardiac irritability following guanidine intoxication and in parathyroid tetany.* O. O. STOLAND and WILBUR POTTER.

The influence of guanidine carbonate and methyl guanidine upon the inhibitory action of the vagus upon the heart was studied in frogs, turtles and dogs.

In frogs and turtles it was shown that the guanidines in sufficient dosage invariably block the inhibitory vagus action upon the heart. This block can easily be removed following a few minutes' washing with Ringer's solution.

In dogs doses of 25 mgm. or less per kilo of body weight injected intravenously generally increased the vagus response to electrical stimulation. The same is true for larger doses injected subcutaneously or very slowly intravenously. Moderate doses 50 to 60 mgm. per kilo body weight in most cases bring on a temporary but marked depression of vagus response to electrical stimulation. This period of depression may be shortened by administration of calcium salts.

Larger doses, 100 mgm. per kilo body weight usually cause profound depression of vagus activity sometimes accompanied by cardiac irregularities.

The irritability of the vagus in dogs suffering from parathyroid tetany was found to vary from normal irritability to fairly marked depression. If this is due to the presence of guanidine or its salts the amount present must be rather large.

To determine whether other parasympathetic mechanisms are affected by guanidine similarly to the vagus we studied their effect upon the chorda tympani. We found that in 17 out of 18 dogs 100 mgm. methyl guanidine sulphate per kilo body weight distinctly increased the secretion of saliva. This secretion is blocked by atropine. Response to electrical stimulation of the chorda tympani following the guanidine injection was lessened in 4 cases, increased in 2 cases and not affected in 2 cases.

Our results indicate that guanidine in small doses increases the irritability of parasympathetic nerves while larger amounts tend to depress the mechanism.

*Changes noted in the specific gravity, hemoglobin and corpuscle count of the blood after profuse sweating.* G. A. TALBERT, R. TYVAND, R. GOEHL and G. GRONHOVD.

The blood was drawn from the basilic vein just before entering the sweat cabinet, and another sample from the opposite arm was taken upon retiring from the same. The Barbour method was used for ascertaining the specific gravity by which it was found that it was higher in the blood after sweating in 68 out of 81 observations. The red count increased as a result of sweating in 65 out of 82 observations. The white count was not so significant as there was an increase noted in only 67 per cent of the observations. The hemoglobin also showed an increase in over 83 per cent of our experiments. Plotted charts are presented made up of the percentage of increase in each observation.

*The effect of the rate of stimulation, strength of the stimulus, summation and reënforcement on the rate of conduction of a nerve impulse through the reflex arc.* W. W. TUTTLE.

The effect of the rate of stimulation, strength of the stimulus, summation and reënforcement on the time elapsing between the application of a stimulus to the Achilles tendon and the arrival of the action current at the gastrocnemius muscle was studied. Data were collected by means of a special apparatus which includes an oscillograph equipped for photographing action currents, an amplifier, an oscillating time device and an automatic stimulating unit.

The data show that the modification of any of the above mentioned factors has no effect on the conduction time through the reflex arc involved in the Achilles-jerk.

*The stimulating effect of ethyl alcohol on the utilization of dextrose, levulose and galactose directly determined.* D. J. VERDA and W. E. BURGE.

The respiratory quotient is the index usually used to the amount of sugar metabolized, a rise in the quotient indicating an increase, and a fall, a decrease in sugar metabolism. In this investigation sugar utilization and the effect of alcohol were determined directly. The body uses alcohol as is indicated by a drop in the respiratory quotient immediately after drinking it. Whether it stimulates the metabolism of other food materials or not seems to be a debated question. The object of this investigation was to determine if ethyl alcohol has any effect on sugar utilization. Two gold fish with a combined weight of approximately five grams were introduced into each of two beakers containing 100 cc. of 0.1 per cent dextrose, levulose and galactose solutions respectively. To one of the beakers containing the dextrose 1 cc. of 95 per cent ethyl alcohol was added while the other beaker to which 1 cc. of water was added served for control. Similarly alcohol was added to the beakers containing the levulose and galactose solutions. Subsequently 0.5 cc. of alcohol were added at intervals of eight hours until 1.5 cc. of alcohol was used in all. Air was kept bubbling through the solutions to insure an adequate supply of oxygen to the fish. It was found that the bubbling of air per se had no effect on sugar utilization. Sugar determinations were run immediately and at the end of thirty hours according to the method of Benedict. In four series of experiments it was found that alcohol produced a large increase in the

utilization of all three of the sugars, the greatest increase being produced in dextrose, the sugar normally used by the body.

*On the sources of energy for cardiac contraction.* M. B. VISSCHER.

Simultaneous studies of the total metabolism of the heart by its oxygen consumption, and the total carbohydrate disappearing, show that when carbohydrate is not abundant, as much as four-fifths of the total metabolism of the heart is non-carbohydrate. The glycogen in the heart muscle is not diminished in amount, even when the free reducing substance has fallen to nearly zero concentration. Some other fuel must be burned in the oxidative recovery processes. The non-protein nitrogen in the system does not increase, consequently reserve protein is probably not the source of energy. There is reason to think that fat can be utilized directly in the metabolism of muscle tissue.

*The behavior of pulmonary vessels as determined by direct observation in the intact chest.* JOSEPH T. WEARN, ARTHUR C. ERNSTENE and A. W. BROMER.

A method has been devised which permits study of the pulmonary circulation in the cat by direct observation and under physiological conditions.

Under amytal anesthesia the parietal pleura of the eighth interspace is exposed in the right anterior axillary line over an area measuring 0.75 by 1.5 cm. Through an abdominal incision a similar pleural window is prepared by removing the muscle fibres of the diaphragm immediately opposite the window in the chest wall. The animal is now curarized, and the lungs are held stationary in the normal inspiratory position by means of a gentle blast of air through a small catheter introduced to the bifurcation of the trachea. By means of the diaphragmatic window a beam of light is passed through a tip of the lower lobe of the lung at sufficient intensity to permit direct observation of the pulmonary vessels with a microscope at the outer window, using a magnification of 110 diameters. Carotid pressure tracings have been made in all experiments. It has been possible with this method to observe the number and calibre of the capillaries and of the smaller arteries and veins. In addition it is possible to detect relative changes in the velocity of blood flow through these vessels.

The number of active capillaries has varied greatly in different preparations. In some instances there have been but one or two capillaries per air sac. In the majority of experiments, however, there have been six to eight capillaries per air sac, while in a few instances the capillaries have been so numerous and have formed such a close-meshed network that accurate counts were impossible. Furthermore, the number of active capillaries in an individual air sac has been observed to vary from time to time. Capillaries have been seen to disappear and reappear without changes in the systemic blood pressure.

Small intravenous doses of adrenalin have been given in a few experiments; and as the systemic blood pressure rose, a variable number of new capillaries has been seen to appear in the air sacs under observation. Further studies upon the effect of this and other drugs are now under way.

*Studies on the visceral vasomotor responses to intravenous injection of purified pancreatic secretin.* MYRON M. WEAVER.

The method of obtaining purified pancreatic secretin was as has been previously described, the process consisting of NaCl precipitation of secretin from acid washings of the duodenum, solution in 90 per cent alcohol and reprecipitation of the active principle by addition of excess ether.

The vasomotor responses to intravenous injection of the secretin were studied in the cases of kidney, spleen, intestine and pancreas.

Kidney volume was followed by oncometer record using balloons in contact with organ and air conduction. The kidney showed negligible increase in volume under the influence of secretin. There was no diuretic effect.

Splenic volume was followed by a similar method, the organ showing no vasomotor change due to the injection of the secretin.

Intestinal vasomotor changes were followed by oncometer and there was no vasomotor response to the injection of purified secretin.

Pancreatic vasomotor changes were studied in a series of dogs by cannulization of the tail vein of that organ after heparization of the animal and direct counting of drops of blood which escaped. There was no observable vasomotor response on the part of the pancreas to intravenous injections of secretin as studied by this method.

*Effect of certain acids and their ammonium salts on metabolism.* MARY WHELAN.

Equivalent amounts of various organic and inorganic acids and their ammonium salts, given to dogs, showed some differences in their effect on metabolism. Since ammonium salts, upon absorption, are very soon ionized with a subsequent synthesis of all or part of the ammonia into urea, the resulting action of the acid ion produces an effect similar to that produced when the acid itself is given.

Observations of the effect of each acid and its ammonium salt upon total base, ammonia, urea, total nitrogen and the inorganic acid ions weremade. No toxic effects were noted from the amounts given.

The organic acids, lactic and acetic and the ammonium salts of these acids, produced no effect on inorganic metabolism. This is presumed to be due to the rapid oxidation of the acid ion into $CO_2$. The inorganic acids caused a distinct lowering of the hydrogen ion concentration of the urine, an evidence of a shift to the acid side of the acid-base equilibrium. Nitric acid and ammonium nitrate produced the most interesting results in that an increased excretion of water and chlorin invariably followed their ingestion.

Special note was made of the response of the ammonia and total base factors called upon to neutralize the acids given.

*Observations on the intracapsular pressure and the molecular concentration of the renal capsular fluid in Necturus.* H. L. WHITE.

1. The pressure in the lumen of the capsules of functioning glomeruli in Necturus was found to range from 1 to 57 mm. of water. The pressure relationships were shown to correspond with those calculated from Poisseuille's law.

2. It was established that Barger's vapor tension method for determining molecular concentration is applicable to blood serum.

3. The molecular concentration of the glomerular fluid was shown to be higher than that of the serum in each of twelve experiments.

4. It is concluded that the glomerular membrane actively secretes a solution more concentrated than the plasma. This process is not incompatible with a simultaneous filtration; it is probable that the latter process is operating, but this has not yet been completely proved.

*Proof that desiccated thyroid increases sugar utilization while commercially prepared thyroxin and other iodine compounds do not.* GEORGE C. WICKWIRE, L. D. SEAGER and W. E. BURGE.

One hundred milligrams of desiccated thyroid containing 0.55 mgm. of iodine were dissolved in 100 cc. of 0.1 per cent dextrose solution. Similarly amounts of thyroxin, calcidine, potassium and sodium iodides containing the same amount of iodine as the 100 mgm. of desiccated thyroid were added to other 100 cc. portions of 0.1 per cent dextrose in beakers. One portion of sugar solution to which nothing was added served for control. Two gold fish with a combined weight of approximately 5 grams were introduced into each beaker. Air was kept bubbling through the sugar solutions to insure an adequate supply of oxygen to the fish. Sugar determinations of the solutions were made immediately and after thirty hours according to the method of Benedict. None of the iodine compounds affected sugar utilization except the desiccated thyroid. This produced an increase of more than 50 per cent over the control. Several experiments similar to the preceding were done with comparable results. Much larger quantities than the above of thyroxin, calcidine, potassium and sodium iodides were used and in no case was utilization increased above the control while smaller quantities of desiccated thyroid gave a marked increase in sugar utilization.

*The static and dynamic effort of the heart beat during ejection.* CARL J. WIGGERS and LOUIS N. KATZ.

The method of studying muscular contraction by measuring the area bounded by tension curves, i.e., by determining their *tension-time values*, cannot be applied directly to the normal intraventricular pressure curve, because the volumes of the ventricles are constantly changing. Some method must, therefore, be discovered, by which the changing capacity factor can be taken into account if *tension-time values* are to be of service in studying afterloaded ventricular contractions in the intact animal.

We report a method by which this is accomplished through a subdivision of the ventricular pressure curve in accordance with the phases of systole and diastole, and present results of such an application to pressure curves recorded under a variety of circulatory conditions.

*Method.* An accurately recorded pressure curve such as shown in figure 1[1] is subdivided by first erecting two perpendiculars so that they cut the pressure curve at the onset and end of ejection respectively and then drawing a horizontal line parallel to the base line from a point representing the pressure level at the onset of ejection. For this report, we shall limit our consideration to *areas B and D* so bounded. We consider *area B* to be a measure of the *static effort* of the ventricle during ejection, in the sense that it represents the pressure energy required to sustain the pressure along a line *b-d* at the diastolic level. We regard *area D* as an index of the

[1] See page. 230, this volume.

dynamic effort, in the sense that it is available for conversion into kinetic energy of flow during some subsequent period of the cardiac cycle. A comparison of the two areas $\left(\text{e.g., as a simple ratio } \frac{D}{B}\right)$ gives an estimate of the economy with which pressure energy is utilized during ejection. Such a comparison is permissible since changes in ventricular volume affect both areas simultaneously and to an equal extent during successive moments of ejection.

*Results.* 1. When heart rate and arterial resistance remain unaltered, an increased venous return increases the *tension-time values* of *areas B and D*. The increase in *area D* is greater, showing that a proportionately larger amount of the extra energy developed is utilized for dynamic functions.

2. When heart rate and the initial tension remain constant, an increased arterial resistance diminishes the size of *areas B and D* combined. The ratio $\frac{D}{B}$ decreases markedly showing that a larger proportion of the entire energy is utilized in the static effort of maintaining the pressure at a high level and that much less is available for dynamic purposes.

3. When the heart rate is constant but the initial stretch and arterial resistance both increase, the effects either neutralize one another or the effect of increased initial stretch predominates somewhat, i.e., the size of *areas B and D* increases to the advantage of *area D*.

4. The economy with which the mechanical energy during ejection is distributed between *areas B and D* at different heart rates depends on the changes in initial stretch: Thus at slow rates, initial stretch is increased. *Areas D and B* therefore increase to the advantage of *area D*. This offers a new insight into the mechanisms which operate to spare the slowly beating heart and to fatigue the rapidly beating one.

*The effect of thyroidectomy and of forcing fluid on the susceptibility to toxins in suprarenalectomized rats.* LELAND C. WYMAN.

It has recently been shown (Corey, Zwemer, 1927) that forcing fluids or thyroidectomy (thereby decreasing elimination of water) prolongs the life of suprarenalectomized cats. Dehydration is one of the symptoms of suprarenal insufficiency in cats. An attempt was made to relieve the symptoms of suprarenal insufficiency in rats by similar methods. In a series of thirty rats which had been completely thyroidectomized one or two weeks previous to double suprarenalectomy 40 per cent died of acute or subacute suprarenal insufficiency within twenty-one days after the latter operation. These animals showed the usual symptoms of acute or subacute insufficiency and at death the usual number showed such findings as diarrhea, bloody nose and eyes, and congested stomach and intestines. Of those which survived ten had gained weight in the period between thyroidectomy and suprarenalectomy but during the three weeks following suprarenalectomy ten lost weight and only three gained. Of these latter one was found at autopsy to have gross accessory cortical tissue. In a series of one hundred and thirty-seven control rats which had been doubly suprarenalectomized without previous treatment 42.3 per cent died of acute or subacute suprarenal insufficiency.

The lethal dose of diphtheria toxin, administered intraperitoneally, averaged 2.75 M.L.D. per gram of body weight both for the thyroidecto-

mized-suprarenalectomized rats and for the suprarenalectomized rats. The lethal dose for normal or blank operated rats averaged 6 M.L.D. per gram of body weight. Both sets of suprarenalectomized rats were, therefore, 2.18 times as susceptible to diphtheria toxin as the controls.

Both thyroidectomized-suprarenalectomized rats and suprarenalectomized rats were killed quite regularly by doses of 7 mgm. of histamine acid phosphate per hundred grams of body weight. All which survived such doses were shown at autopsy to have gross accessory suprarenal tissue. Normal or blank operated rats survived doses of histamine acid phosphate up to 100 mgm. per hundred grams of body weight.

Fluid was administered to rats by means of daily intraperitoneal injections of 10 cc. of a 5 per cent solution of glucose during a period of three to five days at two to four weeks after double suprarenalectomy. Following this treatment the rats were killed by doses of from 5 to 10 mgm. of histamine acid phosphate per hundred grams of body weight. Preliminary experiments indicate that similar administration of glucose solution does not decrease the heightened susceptibility of suprarenalectomized rats to diphtheria toxin.

It appears that thyroidectomy does not decrease the acute and subacute mortality percentage after suprarenalectomy. Also, thyroidectomy or forcing fluid does not decrease the heightened susceptibility of suprarenalectomized rats to the toxins investigated. This suggests that impaired ability to flush toxins out of the body due to dehydration is not an important part of the picture of increased susceptibility.

*The effect of suprarenal insufficiency on the oestrous cycle in the albino rat.* LELAND C. WYMAN.

Of a series of fourteen doubly suprarenalectomized female rats which were exposed to males two weeks after operation seven became pregnant and gave birth to normal litters. Of the seven that did not become pregnant, four died of subacute suprarenal insufficiency within two weeks after exposure to the males. The other three were left with the males for two or three months and were then killed. At autopsy no gross accessory suprarenal tissue was found. In six of the group that had litters gross accessory cortical tissue was found at autopsy. These results suggest that ability to reproduce after double suprarenalectomy is correlated with the degree of suprarenal sufficiency remaining after operation.

In a series of seventeen female rats daily vaginal smears were studied both before and after double suprarenalectomy. Fourteen of this group died of acute or subacute suprarenal insufficiency within twenty-seven days after operation. With one exception these rats showed no oestrous changes in the vaginal smears from the time of operation until death. In the one case the smears were characteristic of oestrus for the seven days preceding death. In the three rats that survived longer there were dioestrous pauses after operation lasting from twenty-one to thirty-six days, following which, oestrous changes appeared at irregular intervals and were separated by much prolonged dioestrous pauses. These rats were exposed to males and two became pregnant. One of these gave birth to a normal litter and at autopsy was found to have a large accessory suprarenal mass; the other died of chronic suprarenal insufficiency on the seventeeth day of pregnancy.

In order to control for operative trauma the vaginal smears were studied as before in a series of twelve rats in which one suprarenal gland was removed and the other gland was exposed and handled. This procedure did not disturb the regular occurrence of vaginal oestrous changes. The remaining suprarenal gland was later removed. Following this, five rats died of acute or subacute suprarenal insufficiency. In two of these there was no change in the occurrence of oestrous changes other than a slight prolongation of the oestrous period. The other three had prolonged dioestrous pauses. Of the seven that survived three showed no significant changes in the occurrence of vaginal oestrous changes and four had occasional much prolonged dioestrous pauses.

Some preliminary experiments involving vital staining of the ovaries indicate that the absence of vaginal oestrous changes after suprarenalectomy is accompanied by absence of ovulation. Work is still in progress.

It appears that in suprarenal insufficiency in the albino rat there may be partial or total inhibition of vaginal oestrous changes presumably due to suppression of ovulation. It is not known whether there is a direct relationship between the functioning of the suprarenal gland and the production of ovarian hormones or an indirect relationship through the mediation of other factors. The irregularity of the effect of suprarenalectomy on the oestrous cycle in the albino rat suggests the latter.